PCT/AU98/00380

# PATENT COOPERATION TREATY

TUESDAY, 19 JAN 1999

ESH

## PCT

From the INTERNATIONAL BUREAU

To:

HUGHES, E., John, L.
Davies Collison Cave
1 Little Collins Street
Melbourne, VIC 3000
AUSTRALIE

### NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

| | |
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| Date of mailing (day/month/year)<br>08 January 1999 (08.01.99) | |
| Applicant's or agent's file reference<br>2049081/EJH | **IMPORTANT NOTIFICATION** |
| International application No.<br>PCT/AU98/00380 | International filing date (day/month/year)<br>22 May 1998 (22.05.98) |

1. The following indications appeared on record concerning:

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| Amrad.<br>THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH<br>300 Herston Road<br>Brisbane, QLD 4029<br>Australia | AU | AU |
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3. Further observations, if necessary:

4. A copy of this notification has been sent to:

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[X] the designated Offices concerned
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| | |
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Form PCT/IB/306 (March 1994)

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WO 98/53061
PCT/AU98/00380

# PATENT COOPERATION TREATY

TUESDAY, - 8 DEC 1998

From the INTERNATIONAL BUREAU

## PCT

**NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES**

(PCT Rule 47.1(c), first sentence)

To:
HUGHES, E., John, L.
Davies Collison Cave
1 Little C llins Street
Melbourne, VIC 3000
AUSTRALIE

**Date of mailing** (day/month/year)
26 November 1998 (26.11.98)

**Applicant's or agent's file reference**
2049081/EJH

**IMPORTANT NOTICE**

| International application No. | International filing date (day/month/year) | Priority date (day/month/year) |
|---|---|---|
| PCT/AU98/00380 | 22 May 1998 (22.05.98) | 23 May 1997 (23.05.97) |

**Applicant**
THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
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In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
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The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 26 November 1998 (26.11.98) under No. WO 98/53061

### REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

### REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

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|---|---|

Form PCT/IB/308 (July 1996)

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**NOTIFICATION OF ELECTION**

(PCT Rule 61.2)

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in its capacity as elected Office

| | |
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| Applicant<br>HAYWARD, Nicholas et al | |

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   04 December 1998 (04.12.98)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was [ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No.: (41-22) 740.14.35 | Authorized officer<br>Lazar Joseph Panakal<br>Telephone No.: (41-22) 338.83.38 |

CORRECTED VERSION*

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International Bureau

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**(54) Title:** THREE NOVEL GENES ENCODING A ZINC FINGER PROTEIN, A GUANINE, NUCLEOTIDE EXCHANGE FACTOR AND A HEAT SHOCK PROTEIN OR HEAT SHOCK BINDING PROTEIN

**(57) Abstract**

The present invention relates generally to three novel human genes with gene regulatory function. These genes encode a zinc finger protein, a guanine nucleotide exchange protein and a heat shock protein or heat shock binding protein. The invention includes derivatives and mammalian animal, insect, nematodes, avian and microbial homologues of these genes. The present invention further provides pharmaceutical compositions and diagnostic agents as well as genetic molecules useful in gene replacement therapy and recombinant molecules useful in protein replacement therapy.

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THREE NOVEL GENES ENCODING A ZINC FINGER PROTEIN, A GUANINE, NUCLEOTIDE EXCHANGE FACTOR AND A HEAT SHOCK PROTEIN OR HEAT SHOCK BINDING PROTEIN

**FIELD OF THE INVENTION**

The present invention relates generally to a novel human gene and its derivatives and to mammalian, animal, insect, nematodes, avian and microbial homologues thereof. The present invention further provides pharmaceutical compositions and diagnostic agents as well as genetic molecules useful in gene replacement therapy and recombinant molecules useful in protein replacement therapy.

**BACKGROUND OF THE INVENTION**

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description.

The increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical and allied health fields. There is growing need to develop recombinant and genetic molecules for use in diagnosis and in conventional pharmaceutical preparations as well as in gene and protein replacement therapies.

In work leading up to the present invention, the inventors sought to identify and clone human genes which might be useful as potential diagnostic and/or therapeutic agents. Molecules of particular interest targeted by the inventors were gene regulators including regulatory proteins, signal transducters and heat shock proteins.

Gene expression generally requires interaction between a regulatory protein and an appropriate recognition sequence of a target gene. Regulatory proteins comprise in many cases a domain or motif which facilitates binding to DNA. One particular motif comprises small sequence units repeated in tandem with each unit folded about a zinc atom to form separate structural domains. This motif is now referred to as a zinc finger domain. Such a domain is generally defined by the number of cysteine (C) and histidine (H) residues.

In addition, knowledge of cellular interaction in the control of cell proliferation is essential in the rational design of specific therapeutic strategies aimed at controlling proliferative disorders. Such proliferative disorders including a range of cancers, inflammatory conditions and atherosclerosis. An important aspect of cellular interaction is in signal transduction *via* receptors to intracellular transducers. One key signal transducer is Ras which couples the receptors for diverse extracellular signals to different effectors. Ras directly activates the downstream kinase Raf which in turn induces the mitogen activated protein kinase (MAPK) cascade.

Another regulatory mechanism involves heat shock proteins. The *Escherichia coli* heat shock protein, DnaJ, is the founding member of a family of proteins which are associated with protein folding, protein complex assembly and transit through subcellular components.

Prokaryotic and eukaryotic DnaJ homologues have a modular organisation consisting of a J domain, a glycine-rich spacer, CXXCXGXG [SEQ ID NO:1] repeats and a C-terminal region with no obvious sequence features, as well as additional sequences for protein targeting. The J domain is anticipated to mediate interaction with heat shock 70 proteins (Hsp70) and consists of some 70 amino acids, frequently located at the N-terminus of the protein.

In accordance with the present invention, a genes have been identified from the human genome which encodes proteins having a regulatory role. One gene, in accordance with the present invention encodes a protein with an N-terminal region resembling a zinc-finger domain of a novel type. Another gene encodes a protein involved in guanine nucleotide exchange factor (GEF) signalling pathways. Yet another gene encodes a protein which is a heat shock protein or heat shock-like protein which may have a role in tumour suppression.

## SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Sequence identity numbers (SEQ ID NOs.) for nucleotide and amino acid sequences referred to in the subject specification are defined after the bibliography. A summary of SEQ ID NOs. is also given in Table 1.

One aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a regulator of gene expression or a derivative of said gene regulator.

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a regulator of gene expression wherein said regulator comprises a zinc finger domain of an $(HC_3)_2$ type.

Yet another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:2;
(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:3;
(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
(iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to the nucleotide sequence set forth in (i), (ii) or (iii).

The nucleotide sequence set forth in SEQ ID NO:2 defines the gene, *mcg4*. This gene encodes a product, MCG4, having an amino acid sequence set forth in SEQ ID NO:3.

Even yet another aspect of the present invention provides a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg4* gene portion, which *mcg4* gene portion is capable of encoding an MCG4 polypeptide or a functional or immunologically interactive derivative thereof.

Still yet another aspect of the present invention contemplates a method of detecting a condition caused or facilitated by an aberration in *mcg4*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg4* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

Even still a further aspect of the present invention relates to a method of detecting a condition caused or facilitated by an aberration in *mcg4*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG4 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

Another aspect of the present invention contemplates a method for detecting MCG4 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG4 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG4 complex to form, and then detecting said complex.

A further aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a guanine nucleotide exchange factor (GEF) or a derivative thereof.

Yet another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:4 or 6;

(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:5 or 7;

(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and

(iv) a nucleotide sequence capable of hybridizing under low stringency conditions to the

nucleotide sequence set forth in (i), (ii) or (iii).

The nucleotide sequence set forth in SEQ ID NO:4 or 6 defines the gene, *mcg7*. This gene encodes a product, MCG7, having an amino acid sequence set forth in SEQ ID NO:5 or 7.

Even yet another aspect of the present invention provides a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg7* gene portion, which *mcg7* gene portion is capable of encoding an MCG7 polypeptide or a functional or immunologically interactive derivative thereof.

Still yet another aspect of the present invention contemplates a method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg7* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

Even still a further aspect of the present invention relates to a method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG7 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

Another aspect of the present invention contemplates a method for detecting MCG7 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG7 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG7 complex to form, and then detecting said complex.

Yet another aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a heat shock protein or a heat shock binding protein or a derivative thereof.

Another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:8;
(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:9;
(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
(iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 41°C to the nucleotide sequence set forth in (i), (ii) or (iii).

The nucleotide sequence set forth in SEQ ID NO:8 defines the gene, *mcg18*. This gene encodes a product, MCG18, having an amino acid sequence set forth in SEQ ID NO:7.

Even yet another aspect of the present invention provides a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg18* gene portion, which *mcg18* gene portion is capable of encoding an MCG18 polypeptide or a functional or immunologically interactive derivative thereof.

Still yet another aspect of the present invention contemplates a method of detecting a condition caused or facilitated by an aberration in *mcg18,* said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg18* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

Even still a further aspect of the present invention relates to a method of detecting a condition caused or facilitated by an aberration in *mcg18,* said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG18 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

Another aspect of the present invention contemplates a method for detecting MCG18 or a

derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG18 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG18 complex to form, and then detecting said complex.

A summary of SEQ ID Nos. referred to in the subject specification is shown in Table 1.

**TABLE 1**

**SUMMARY OF SEQ ID Nos.**

| SEQ ID NO. | DESCRIPTION |
|---|---|
| 1 | amino acid repeat sequence in DnaJ homologues |
| 2 | Nucleotide sequence of *mcg4* |
| 3 | amino acid sequence of MCG4 |
| 4 | nucleotide sequence of *mcg7* |
| 5 | amino acid sequence of MCG7 |
| 6 | nucleotide sequence of *mcg7* within exon of nucleotides 183-288 |
| 7 | amino acid sequence of MCG7 within exon of nucleotide 183-288 |
| 8 | nucleotide sequence of *mcg18* |
| 9 | amino acid sequence of MCG18 |
| 10-18 | amino acid sequence identified using BESTFIT |
| 19 | sequence of pGEX and *mcg7* junction |
| 20 | sequence of pGEX and *mcg7* junction |
| 21 | nucleotide sequence of *myc*-tag/*mcg7* junction |
| 22 | amino acid sequence corresponding to SEQ ID NO:21 |
| 23 | nucleotide sequence of pGEX and *mcg7* junction |
| 24 | amino acid sequence corresponding to SEQ ID NO:23 |
| 25-36 | *mcg7*-specific oligonucleotide |
| 37-45 | *mcg18*-specific oligonucleotide |

Single and three letter abbreviations for amino acid residues are shown in Table 2.

**TABLE 2**

| Amino Acid | Three-letter Abbreviation | One-letter Symbol |
|---|---|---|
| Alanine | Ala | A |
| Arginine | Arg | R |
| Asparagine | Asn | N |
| Aspartic acid | Asp | D |
| Cysteine | Cys | C |
| Glutamine | Gln | Q |
| Glutamic acid | Glu | E |
| Glycine | Gly | G |
| Histidine | His | H |
| Isoleucine | Ile | I |
| Leucine | Leu | L |
| Lysine | Lys | K |
| Methionine | Met | M |
| Phenylalanine | Phe | F |
| Proline | Pro | P |
| Serine | Ser | S |
| Threonine | Thr | T |
| Tryptophan | Trp | W |
| Tyrosine | Tyr | Y |
| Valine | Val | V |
| Any residue | Xaa | X |

**BRIEF DESCRIPTION OF THE FIGURES**

**Figure 1** is a representation of the nucleotide sequence [SEQ ID NO:2] and corresponding amino acid sequence [SEQ ID NO:3] of *mcg4*.

**Figure 2** is a representation of the alignment of the human MCG4 amino acid sequence with a translation of a partial murine expressed sequence tag (EST).

**Figure 3** is a representation of the alignment of the human MCG4 amino acid sequence with a translation of a partial nematode EST.

**Figure 4** is a diagrammatic representation showing a predicted structure of MCG4 where H and C represent histidine and cysteine residues, respectively and X refers to any amino acid residue. Zn represent zinc atoms.

**Figure 5** is a representation of sensitive sequence homology search of related cysteine-containing motifs in another *Caenorhabditis elegans* protein.

**Figure 6** is a representation showing that a related cysteine containing motif is present in the GATA-binding transcription factor from *Saccharomyces pombe*.

**Figure 7** is a Northern blot showing expression of *mcg4* in various cultured human cancer cell lines. Lanes 1-5, respectively, represent the hybridization signal from 15$\mu$g total RNA derived from various human cancer cell lines. Lanes 1-5, respectively, contain RNA from H69 lung carcinoma cells, JAM ovary carcinoma cells, BT20 breast carcinoma cells, HaCat transformed keratinocytes, T24 bladder carcinoma cells.

**Figure 8** is a representation of a partial alignment of *mcg4* with human ESTs AA074703 and AA134788.

**Figure 9** is a representation of the partial nucleotide sequence alignment between a human

(W32939) and mouse (AA242159) *mcg4*-like EST in the putative 5′ UTR of the *mcg4* cDNA. The putative initiation codon is underlined and the region upstream represents 5′ UTR.

**Figure 10** is a representation showing MacVector alignment of MCG4 with forward translations of ESTs AA134788 and AA074703. The nucleotide sequences are shown in Figure 8.

**Figure 11** is a diagrammatic representation of the domains of MCG4

zinc finger consensus: $CX_2HX_4CX_2CX_4HX_2CX_{17}CX_2CX_{18}HX_2CX_{18}CX_2C$

acidic domain consensus: 9/34 amino acids negatively charged, 0/34 positively charged

basic domain consensus: 13/55 amino acids positively charged, 0/55 negatively charged

leucine zipper domain consensus: $LX_6LX_6RX_6LX_6L$

alternate "novel" leucine zipper-like motif where leucine would not be aligned along the one surface of an alpha helix domain: (aa261) $LX_6LXLX_6LXLX_6L$ (aa 286).

**Figure 12** is a representation showing similarity of MCG7 with GEFs of various organisms.

**Figure 13(a)** is a representation of the nucleotide sequence [SEQ ID NO:4] and corresponding amino acid sequence [SEQ ID NO:5] of *mcg7*. Nucleotides 183-288 are an alternative spliced exon (shown in lower case).

**Figure 13(b)** is a representation of the partial nucleotide sequence [SEQ ID NO:6] and corresponding amino acid sequence [SEQ ID NO:7] of *mcg7* but without the exon shown in Fig. 13(a). Amino acids have been numbered from the first methionine codon (underlined). The cDNA molecules of Fig. 13(a) and Fig. 13(b) differ by the inclusion and exclusion of the exon of nucleotides 183-288.

**Figure 14** is a representation showing a comparison between MCG7 and a homologue from *Caenorhabditis elegans* using the BESTFIT algorithm. in the figure, the following sequences are underlined:

**EF-Hand= PROSITE DATABASE NO. PD0C00018**

| | | |
|---|---|---|
| 1a | nematode | DVDEEDEVEDIEF [SEQ ID NO:10] |
| 1b | human | DVDGDGHISQEEF [SEQ ID NO:11] |
| | nematode | DHDRDGFISQEEF [SEQ ID NO:12] |
| 1c | human | DQNQDGCISREEM [SEQ ID NO:13] |
| | nematode | DVDMDGQISKDEL [SEQ ID NO:14] |

**GUANINE NT BINDING REGION = BLOCKS DATABASE NO. BL00720B**

| | | |
|---|---|---|
| 2 | human | HFVHVAEKLLQLQNFNTLMAVVGGLSHSSISRLKETH [SEQ ID NO:15] |
| | nematode | KFVHVAKHLRKINNFNTLMSVVGGITHSSVARLAKTY [SEQ ID NO:16] |

**DaG-PE BINDING DOMAIN = PROSITE DATABASE NO. PD0C00379**

| | | |
|---|---|---|
| 3 | human | HNFQESNSLRPVACRHCKALILGIYKQGLKCRACGVNCHKQCKDRLSVEC [SEQ ID NO:17] |
| | nematode | HNFHETTFLTPTTCNHCNKLLWGILRQGFKCKDCGLAVHSCCKSNAVAEC [SEQ ID NO:18] |

**Figure 15** is a representation of an alignment of human and a partial (5′ UTR and partial coding sequence) murine *mcg*7 cDNA (GenBank Acc. No. W71787 and AA237373). The putative initiation codon is underlined. The murine sequence represents a composite of 2 partial cDNA sequences from the EST database (accession numbers W71787 and AA237373). Nucleotide differences between human and murine sequences are shown in lower case lettering and identical residues are indicated with asterisks.

**Figure 16** is a representation of further 5' nucleotide and corresponding amino acid sequence for human *mcg*7. Nucleotide positions 1-321 were derived from GenBank Acc. No. AC000134 and nucleotides 322 onwards from Fig. 13(a). Two in-frame initiation codons are underlined. Asterisks denote in-frame stop codons.

**Figure 17** is a graphical representation of a GDP release assay. □ Experiment #1 (mean of duplicates). ◇ Experiment #2 (mean of duplicates). The exchange reaction contained 36pmols

of GST-MCG (N-terminally truncated; encoded by Construct B in Fig. 18) and 1.6-12.8 pmols of recombinant GST-N-Ras.GDP. Reaction time 6 mins.

Estimated reaction constants:

$K_m$ = 2.1μM, $V_{max}$ = 37pMol/6min/36pMol [Expt#1]

$K_m$ = 1.5μM, $V_{max}$ = 30.3pMol/6 min/36pMol [Expt#2]

**Figure 18** depicts various recombinant plasmids containing partial or full-length *mcg7.*

**Figure 19** is a representation of the nucleotide sequence [SEQ ID NO:8] and corresponding amino acid sequence [SEQ ID NO:9] of *mcg18.*

**Figure 20** is a representation showing that MCG18 has partial homology to *E. coli* DnaJ.

**Figure 21** is a representation showing that MCG18 has homology to two *Caenorhabitis elegans* proteins.

**Figure 22** is a representation showing that MCG18 has homology to a *Saccharomyces pombe* protein.

**Figure 23** is a representation showing homology of MCG18 to a *Drosophila virilis* protein.

**Figure 24** is a representation showing homology of MCG18 to human DnaJ proteins HDJ-2/HSDJ, HDJ-1/HSP40 and HSJ1.

**Figure 25** is a representation of the nucleotide and corresponding amino acid sequence of murine *mcg18.*

**Figure 26** is a representation of homology between human and murine MCG18.

**Figure 27** depicts nucleotide sequences corresponding to the 5′ untranslated region of human *mcg18.*

**Figure 28** depicts a Northern blot showing expression of *mcg18* transcripts in total RNA isolated from various human cancer cell lines grown in culture. Lanes 1-5 respectively contain 15$\mu$g RNA from H69 lung carcinoma cells, JAM ovary carcinoma cells, BT20 breast carcinoma cells, HaCat transformed keratinocytes, T24 bladder carcinoma cells.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT**

The present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a regulator of gene expression or a derivative of said gene regulator.

More particularly, the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a regulator of gene expression wherein said regulator comprises a zinc finger domain of an $(HC_3)_2$ type.

Still more particularly, the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:2;
(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:3;
(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
(iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to the nucleotide sequence set forth in (i), (ii) or (iii).

The present invention also provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a guanine nucleotide exchange factor (GEF) or a derivative thereof.

More particularly, the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:4 or 6;
(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:5 or 7;

(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and

(iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to the nucleotide sequence set forth in (i), (ii) or (iii).

Another aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a heat shock protein or a heat shock-binding protein or a derivative thereof.

More particularly, the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:8;

(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:9;

(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and

(iv) a nucleotide sequence capable of hybridizing under low stringency conditions at 42°C to the nucleotide sequence set forth in (i), (ii) or (iii).

Preferably, the percentage similarity is at least about 50%. More preferably, the percentage similarity is at least about 60%.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least

about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels.

The present invention extends to nucleic acid molecules with percentage similarities of approximately 65%, 70%, 75%, 80%, 85%, 90% or 95% or above or a percentage in between.

The nucleic acid molecule of the present invention defined by SEQ ID NO:2 is hereinafter referred to as constituting the "*mcg4*" gene. The protein encoded by *mcg4* is referred to herein as "MCG4"and has an amino acid sequence set forth in SEQ ID NO:3. The *mcg4* gene is proposed to encode, in accordance with the present invention, a regulator of gene expression and comprises a novel zinc finger domain, $(HC_3)_2$. A regulator of gene expression includes a transcription factor. Regulation may be at the level of nucleic acid:protein or protein:protein interaction.

The nucleic acid molecule of the present invention defined by SEQ ID NO:4 or 6 is hereinafter referred to as constituting the "*mcg7*" gene. The protein encoded by *mcg7* is referred to herein as "MCG7" and has an amino acid sequence set forth in SEQ ID NO:5 or 7 and is involved in signal transduction. The difference in the nucleotide and amino acid sequence is due to the presence or absence of an exon at nucleotides 183-288.

The nucleic acid molecule of the present invention defined by SEQ ID NO:8 is hereinafter referred to as constituting the "*mcg18*" gene. The protein encoded by *mcg18* is referred to herein as "MCG18" and comprises the amino acid set forth in SEQ ID NO:9.

The present invention extends to the naturally occurring genomic *mcg4, mcg7* and *mcg18* nucleotide sequences or corresponding cDNA sequences or to derivatives thereof. Derivatives contemplated in the present invention include fragments, parts, portions, mutants, homologues and analogues of MCG4, MCG7 or MCG8 or the corresponding genetic sequences. Derivatives also include single or multiple amino acid substitutions, deletions and/or additions to MCG4, MCG7 or MCG18 or single or multiple nucleotide substitutions, deletions and/or additions to *mcg4, mcg7* or *mcg18*. "Additions" to the amino acid or nucleotide sequences include fusions with other peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to "MCG4" or "*mcg4*", "MCG7" or "*mcg7*" or "MCG8" or *mcg18*" includes reference to all derivatives thereof including functional derivatives and immunologically interactive derivatives of MCG4, MCG7 or MCG18.

The *mcg4, mcg7* and *mcg18* of the present invention are particularly exemplified herein from humans and in particular from human chromosome 11q13.

The present invention extends, however, to a range of homologues from, for example, primates, livestock animals (eg. sheep, cows, horses, donkeys, pigs), companion animals (eg. dogs, cats) laboratory test animals (eg. rabbits, mice, rats, guinea pigs), reptiles, birds (eg. chickens, ducks, geese, parrots), insects, nematodes, eukaryotic microorganisms and captive wild animals (eg. deer, foxes, kangaroos). Reference herein to *mcg4* and *mcg18* or their respective proteins MCG4, MCG7 and MCG18 includes reference to these molecules of human origin as well as novel forms of non-human origin.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or

both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli, Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg4* gene portion, which *mcg4* gene portion is capable of encoding an MCG4 polypeptide or a functional or immunologically interactive derivative thereof.

Preferably, the *mcg4* gene portion of the genetic construct is operably linked to a promoter in the vector such that said promoter is capable of directing expression of said *mcg4* gene portion in an appropriate cell.

In addition, the *mcg4* gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

It is proposed in accordance with the present invention that MCG4 is a transcription factor involved in gene regulation. Mutations in *mcg4* may result in aberrations in gene regulation leading to the development of or a propensity to develop various types of cancer. In this regard, although not wishing to limit the present invention to any one hypothesis or mode of action, it is proposed that *mcg4* or its expression product may be involved in the tissue-specific or temporal regulation of particular genes.

A deletion or aberration in the *mcg4* gene may also be important in the detection of cancer or a propensity to develop cancer. An aberration may be a homozygous mutation or a heterozygous mutation. The detection may occur at the foetal or post-natal level. Detection may also be at the germline or somatic cell level. Furthermore, a risk of developing cancer may

be determined by assaying for aberrations in the parents and/or proband of a subject under investigation.

According to this aspect of the present invention, there is contemplated a method of detecting a condition caused or facilitated by an aberration in *mcg4*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg4* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

Another aspect of the present invention contemplates a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg7* gene portion, which *mcg7* gene portion is capable of encoding an *mcg7* polypeptide or a functional or immunologically interactive derivative thereof.

Preferably, the *mcg7* gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said *mcg7* gene portion in an appropriate cell.

In addition, the *mcg7* gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

It is proposed in accordance with the present invention that MCG7 is a GEF involved in signal transduction. Mutations in *mcg7* or MCG7 may result in defective control of cell proliferation leading to the development of or a propensity to develop various types of cancer.

A deletion or aberration in the *mcg7* gene may also be important in the detection of cancer or

a propensity to develop cancer. An aberration may be a homozygous mutation or a heterozygous mutation. The detection may occur at the foetal or post-natal level. Detection may also be at the germline or somatic cell level. Furthermore, a risk of developing cancer may be determined by assaying for aberrations in the parents of a subject under investigation.

According to this aspect of the present invention, there is contemplated a method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg7* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

Yet another aspect of the present invention contemplates a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg18* gene portion, which *mcg18* gene portion is capable of encoding an MCG18 polypeptide or a functional or immunologically interactive derivative thereof.

Preferably, the *mcg18* gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said *mcg18* gene portion in an appropriate cell.

In addition, the *mcg18* gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

It is proposed in accordance with the present invention that MCG18 is a transcription factor involved in protein folding, protein complex assembly and transit through subcellular compartments. MCG18 may also have a role in tumour suppression. Thus mutations in *mcg18*

may result in the development of or a propensity to develop various types of cancer.

A deletion or aberration in the *mcg18* gene may also be important in the detection of cancer or a propensity to develop cancer. An aberration may be a homozygous mutation or a heterozygous mutation. The detection may occur at the foetal or post-natal level. Detection may also be at the germline or somatic cell level. Furthermore, a risk of developing cancer may be determined by assaying for aberrations in the parents and/or proband of the subject under investigation.

According to this aspect of the present invention, there is contemplated a method of detecting a condition caused or facilitated by an aberration in *mcg18*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg18* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

The nucleotide substitutions, additions or deletions may be detected by any convenient means including nucleotide sequencing, restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), oligonucleotide hybridization and single stranded conformation polymorphism analysis (SSCP) amongst many others. An aberration includes modification to existing nucleotides such as to modify glycosylation signal amongst other effects.

In an alternative method, aberrations in the *mcg4*, *mcg7* and *mcg18* genes are detected by screening for mutations in MCG4, MCG7 and MCG18, respectively.

A mutation in MCG4, MCG7 or MCG18 may be a single or multiple amino acid substitution, addition and/or deletion. The mutation in *mcg4*, *mcg7* or *mcg18* may also result in either no translation product being produced or a product in truncated form. A mutant may also be an altered glycosylation pattern or the introduction of side chain modifications to amino acid residues.

According to this aspect of the present invention, there is provided a method of detecting a condition caused or facilitated by an aberration in *mcg4, mcg7* or *mcg18* said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG4, MCG7 or MCG18 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

A particularly convenient means of detecting a mutation in MCG4, MCG7 or MCG18 is by use of antibodies.

Accordingly another aspect of the present invention is directed to antibodies to MCG4, MCG7 or MCG18 and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to MCG4, MCG7 or MCG18 or may be specifically raised to MCG4, MCG7 or MCG18 or derivatives thereof. In the case of the latter, MCG4, MCG7 or MCG18 or their derivatives may first need to be associated with a carrier molecule. The antibodies to MCG4, MCG7 or MCG18 of the present invention are particularly useful as diagnostic agents.

For example, antibodies to MCG4, MCG7 or MCG18 and their derivatives can be used to screen for wild-type MCG4, MCG7 or MCG18 or for mutated MCG4, MCG7 or MCG18 molecules. The latter may occur, for example, during or prior to certain cancer development. A differential binding assay is also particularly useful. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of normal MCG4, MCG7 or MCG18 levels or the presence of wild-type MCG4, MCG7 or MCG18 may be important for diagnosis of certain cancers or a predisposition for development of cancers or for monitoring certain therapeutic protocols.

As stated above antibodies to MCG4, MCG7 or MCG18 of the present invention may be monoclonal or polyclonal or may be fragments of antibodies such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies.

For example, specific antibodies can be used to screen for wild-type MCG4, MCG7 or MCG18 molecule or specific mutant molecules such as molecules having a certain deletion. This would be important, for example, as a means for screening for levels of MCG4, MCG7 or MCG18 in a cell extract or other biological fluid or purifying MCG4, MCG7 or MCG18 made by recombinant means from culture supernatant fluid or purified from a cell extract. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of wild-type MCG4, MCG7 or MCG18 or to a specific mutant phenotype or to a deleted or otherwise altered region.

Both polyclonal and monoclonal antibodies are obtainable by immunization of a suitable animal or bird with MCG4, MCG7 or MCG18 or its derivatives and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal or bird with an effective amount of MCG4, MCG7 or MCG18 or antigenic parts thereof or derivatives thereof, collecting serum from the animal or bird, and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates a method for detecting MCG4, MCG7 or MCG18 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG4, MCG7 or MCG18 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG4, MCG7 or MCG18 complex to form, and then detecting said complex.

Preferably, the biological sample is a cell extract from a human or other animal or a bird.

The presence of MCG4, MCG7 or MCG18 may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain MCG4, MCG7 or MCG18 including cell extract

or tissue biopsy. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

In the typical forward sandwich assay, a first antibody having specificity for the MCG4, MCG7 or MCG18 or an antigenic part thereof or a derivative thereof or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to 37°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide

containing molecules (i.e. radioisotopes) and chemiluminescent molecules.
In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

As stated above, the present invention extends to genetic constructs capable of encoding MCG4,

MCG7 or MCG18 or functional derivatives thereof. Such genetic constructs are also contemplated to be useful in modulating expression of specific genes in which *mcg4, mcg7* or *mcg18* is involved in tissue-specific or temporal regulation.

Accordingly, another aspect of the present invention is directed to a genetic construct comprising a nucleotide sequence encoding a peptide, polypeptide or protein and *mcg4, mcg7* or *mcg18* or a functional derivative or homologue thereof capable of modulating the expression of said nucleotide sequence.

As stated above, MCG18 is proposed to have a role in tumour suppression. Accordingly, it is further proposed in accordance with the present invention to use recombinant MCG18 in pharmaceutical preparations for treating arresting or otherwise ameliorating the effects of certain cancers.

Accordingly, another aspect of the present invention contemplates a method for treating, arresting or otherwise ameliorating the effects of a cancer in an animal or bird, said method comprising administering to said animal or bird an effective amount of MCG18 or a functional derivative thereof for a time and under conditions sufficient to treat, arrest or otherwise ameliorate the effects of said cancer.

The present invention, therefore, contemplates a pharmaceutical composition comprising MCG18 or a derivative thereof or a modulator of *mcg18* expression or MCG18 activity and one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to hereinafter as the "active ingredients". The active ingredients may also include anti-cancer agents or agents which facilitate actions of MCG18.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier may be a solvent medium containing, for example, water, ethanol, polyol (for example,

glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 $\mu$g and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter. A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium

phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the

treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

Effective amounts contemplated by the present invention include those amounts effective to ameliorate a condition. For example, it is envisaged that effective amounts would range from about 0.001 μg/kg body weight to about 100 mg/kg body weight. Alternatively, effective amounts of about 0.01 μg/kg body weight to about 10 mg/kg body weight or even 0.1 μg/kg body weight to about 1 mg/kg body weight. Administration may be per minute, hour, day, week, month or year or may only be a once off administration.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating *mcg18* expression or MCG18 activity. The vector may, for example, be a viral vector.

As stated above, the present invention further contemplates a range of derivatives of MCG18. Derivatives include fragments, parts, portions, mutants, homologues and analogues of the MCG18 polypeptide and corresponding genetic sequence. Derivatives also include single or multiple amino acid substitutions, deletions and/or additions to MCG18 or single or multiple nucleotide substitutions, deletions and/or additions to the genetic sequence encoding MCG18. "Additions" to amino acid sequences or nucleotide sequences include fusions with other peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to "MCG18" includes reference to all derivatives thereof including functional derivatives or MCG18 immunologically interactive derivatives.

Analogues of MCG18 contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids, contemplated herein is shown in Table 3.

**TABLE 3**

| Non-conventional amino acid | Code | Non-conventional amino acid | Code |
|---|---|---|---|
| α-aminobutyric acid | Abu | L-N-methylalanine | Nmala |
| α-amino-α-methylbutyrate | Mgabu | L-N-methylarginine | Nmarg |
| aminocyclopropane-carboxylate | Cpro | L-N-methylasparagine | Nmasn |
| | | L-N-methylaspartic acid | Nmasp |
| aminoisobutyric acid | Aib | L-N-methylcysteine | Nmcys |
| aminonorbornyl-carboxylate | Norb | L-N-methylglutamine | Nmgln |
| | | L-N-methylglutamic acid | Nmglu |
| cyclohexylalanine | Chexa | L-N-methylhistidine | Nmhis |
| cyclopentylalanine | Cpen | L-N-methylisolleucine | Nmile |
| D-alanine | Dal | L-N-methylleucine | Nmleu |
| D-arginine | Darg | L-N-methyllysine | Nmlys |
| D-aspartic acid | Dasp | L-N-methylmethionine | Nmmet |
| D-cysteine | Dcys | L-N-methylnorleucine | Nmnle |
| D-glutamine | Dgln | L-N-methylnorvaline | Nmnva |
| D-glutamic acid | Dglu | L-N-methylornithine | Nmorn |
| D-histidine | Dhis | L-N-methylphenylalanine | Nmphe |
| D-isoleucine | Dile | L-N-methylproline | Nmpro |
| D-leucine | Dleu | L-N-methylserine | Nmser |
| D-lysine | Dlys | L-N-methylthreonine | Nmthr |
| D-methionine | Dmet | L-N-methyltryptophan | Nmtrp |
| D-ornithine | Dorn | L-N-methyltyrosine | Nmtyr |
| D-phenylalanine | Dphe | L-N-methylvaline | Nmval |
| D-proline | Dpro | L-N-methylethylglycine | Nmetg |
| D-serine | Dser | L-N-methyl-t-butylglycine | Nmtbug |
| D-threonine | Dthr | L-norleucine | Nle |
| D-tryptophan | Dtrp | L-norvaline | Nva |

| | | | |
|---|---|---|---|
| D-tyrosine | Dtyr | α-methyl-aminoisobutyrate | Maib |
| D-valine | Dval | α-methyl-γ-aminobutyrate | Mgabu |
| D-α-methylalanine | Dmala | α-methylcyclohexylalanine | Mchexa |
| D-α-methylarginine | Dmarg | α-methylcylcopentylalanine | Mcpen |
| D-α-methylasparagine | Dmasn | α-methyl-α-napthylalanine | Manap |
| D-α-methylaspartate | Dmasp | α-methylpenicillamine | Mpen |
| D-α-methylcysteine | Dmcys | N-(4-aminobutyl)glycine | Nglu |
| D-α-methylglutamine | Dmgln | N-(2-aminoethyl)glycine | Naeg |
| D-α-methylhistidine | Dmhis | N-(3-aminopropyl)glycine | Norn |
| D-α-methylisoleucine | Dmile | N-amino-α-methylbutyrate | Nmaabu |
| D-α-methylleucine | Dmleu | α-napthylalanine | Anap |
| D-α-methyllysine | Dmlys | N-benzylglycine | Nphe |
| D-α-methylmethionine | Dmmet | N-(2-carbamylethyl)glycine | Ngln |
| D-α-methylornithine | Dmorn | N-(carbamylmethyl)glycine | Nasn |
| D-α-methylphenylalanine | Dmphe | N-(2-carboxyethyl)glycine | Nglu |
| D-α-methylproline | Dmpro | N-(carboxymethyl)glycine | Nasp |
| D-α-methylserine | Dmser | N-cyclobutylglycine | Ncbut |
| D-α-methylthreonine | Dmthr | N-cycloheptylglycine | Nchep |
| D-α-methyltryptophan | Dmtrp | N-cyclohexylglycine | Nchex |
| D-α-methyltyrosine | Dmty | N-cyclodecylglycine | Ncdec |
| D-α-methylvaline | Dmval | N-cylcododecylglycine | Ncdod |
| D-N-methylalanine | Dnmala | N-cyclooctylglycine | Ncoct |
| D-N-methylarginine | Dnmarg | N-cyclopropylglycine | Ncpro |
| D-N-methylasparagine | Dnmasn | N-cycloundecylglycine | Ncund |
| D-N-methylaspartate | Dnmasp | N-(2,2-diphenylethyl)glycine | Nbhm |
| D-N-methylcysteine | Dnmcys | N-(3,3-diphenylpropyl)glycine | Nbhe |
| D-N-methylglutamine | Dnmgln | N-(3-guanidinopropyl)glycine | Narg |
| D-N-methylglutamate | Dnmglu | N-(1-hydroxyethyl)glycine | Nthr |
| D-N-methylhistidine | Dnmhis | N-(hydroxyethyl))glycine | Nser |
| D-N-methylisoleucine | Dnmile | N-(imidazolylethyl))glycine | Nhis |
| D-N-methylleucine | Dnmleu | N-(3-indolylethyl)glycine | Nhtrp |

| | | | |
|---|---|---|---|
| D-N-methyllysine | Dnmlys | N-methyl-γ-aminobutyrate | Nmgabu |
| N-methylcyclohexylalanine | Nmchexa | D-N-methylmethionine | Dnmmet |
| D-N-methylornithine | Dnmorn | N-methylcyclopentylalanine | Nmcpen |
| N-methylglycine | Nala | D-N-methylphenylalanine | Dnmphe |
| N-methylaminoisobutyrate | Nmaib | D-N-methylproline | Dnmpro |
| N-(1-methylpropyl)glycine | Nile | D-N-methylserine | Dnmser |
| N-(2-methylpropyl)glycine | Nleu | D-N-methylthreonine | Dnmthr |
| D-N-methyltryptophan | Dnmtrp | N-(1-methylethyl)glycine | Nval |
| D-N-methyltyrosine | Dnmtyr | N-methyla-napthylalanine | Nmanap |
| D-N-methylvaline | Dnmval | N-methylpenicillamine | Nmpen |
| γ-aminobutyric acid | Gabu | N-(*p*-hydroxyphenyl)glycine | Nhtyr |
| L-*t*-butylglycine | Tbug | N-(thiomethyl)glycine | Ncys |
| L-ethylglycine | Etg | penicillamine | Pen |
| L-homophenylalanine | Hphe | L-α-methylalanine | Mala |
| L-α-methylarginine | Marg | L-α-methylasparagine | Masn |
| L-α-methylaspartate | Masp | L-α-methyl-*t*-butylglycine | Mtbug |
| L-α-methylcysteine | Mcys | L-methylethylglycine | Metg |
| L-α-methylglutamine | Mgln | L-α-methylglutamate | Mglu |
| L-α-methylhistidine | Mhis | L-α-methylhomophenylalanine | Mhphe |
| L-α-methylisoleucine | Mile | N-(2-methylthioethyl)glycine | Nmet |
| L-α-methylleucine | Mleu | L-α-methyllysine | Mlys |
| L-α-methylmethionine | Mmet | L-α-methylnorleucine | Mnle |
| L-α-methylnorvaline | Mnva | L-α-methylornithine | Morn |
| L-α-methylphenylalanine | Mphe | L-α-methylproline | Mpro |
| L-α-methylserine | Mser | L-α-methylthreonine | Mthr |
| L-α-methyltryptophan | Mtrp | L-α-methyltyrosine | Mtyr |

| | | | |
|---|---|---|---|
| L-$\alpha$-methylvaline | Mval | L-N-methylhomophenylalanine | Nmhphe |
| N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine | Nnbhm | N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine | Nnbhe |
| 1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane | Nmbc | | |

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of $C_\alpha$ and $N_\alpha$-methylamino acids, introduction of double bonds between $C_\alpha$ and $C_\beta$ atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

Such analogues also apply in respect of MCG4 and MCG7.

The present invention further contemplates chemical analogues of MCG18 capable of acting as antagonists or agonists of MCG18 or which can act as functional analogues of MCG18. Chemical analogues may not necessarily be derived from MCG18 but may share certain conformational similarities. Alternatively, chemical analogues may be specifically designed to mimic certain physiochemical properties of MCG18. Chemical analogues may be chemically synthesised or may be detected following, for example, natural product screening.

The identification of MCG18 permits the generation of a range of therapeutic molecules capable of modulating expression of MCG18 or modulating the activity of MCG18. Modulators contemplated by the present invention includes agonists and antagonists of MCG18 expression. Antagonists of MCG18 expression include antisense molecules, ribozymes and co-suppression

molecules. Agonists include molecules which increase promoter ability or interfere with negative regulatory mechanisms. Agonists of MCG18 include molecules which overcome any negative regulatory mechanism. Antagonists of MCG18 include antibodies and inhibitor peptide fragments.

These types of modifications may be important to stabilise MCG18 if administered to an individual or for use as a diagnostic reagent.

Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

Another embodiment of the present invention contemplates a method for modulating expression of MCG18 in a human, said method comprising contacting the *mcg18* gene encoding MCG18 with an effective amount of a modulator of *mcg18* expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *mcg18*. For example, a nucleic acid molecule encoding MCG18 or a derivative thereof may be introduced into a cell to facilitate protection of that cell from becoming cancerous.

Another aspect of the present invention contemplates a method of modulating activity of MCG18 in a human, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease MCG18 activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative of MCG18 or a chemical analogue or truncation mutant of MCG18.

The present invention is further described with reference to the following non-limiting Examples.

## EXAMPLE 1

A human gene (designated *mcg4*) was identified on chromosome 11q13 that on the basis of sequence homology is predicted to encode a putative transcription factor of 310 amino acids (Fig. 1). *mcg4* is transcribed in several different cell lines (Fig. 7).

## EXAMPLE 2

The expressed sequence tag (EST) database contains partial sequence data for the murine (Fig. 2) and nematode (Fig. 3) homologues of *mcg4*.

## EXAMPLE 3

MCG4 contains a sequence of cysteine residues within the N-terminal region of the protein that resembles zinc-finger binding domains of a novel type, ie. $(HC_3)_2$ [Fig. 4].

## EXAMPLE 4

Sensitive sequence homology searches reveal that related cysteine-containing motifs are present in another *C. elegans* protein (Fig. 5) as well as the GATA-binding transcription factor from *S. pombe* (Fig. 6).

## EXAMPLE 5

*mcg4* will have commercial value due to its likelihood of encoding a novel transcription factor that is highly conserved amongst organisms, thus suggesting an integral role in gene regulation. *mcg4* may also be involved in some way in tissue-specific or temporal regulation of certain genes, thus making it a potential target for modulating expression of those downstream effectors.

## EXAMPLE 6

Nucleotide sequence data generated from cosmid clone cSRL-72c4 with the T7 primer (Promega, and Applied Biosystems Incorporated dye terminator sequencing kit) was aligned to the GenBank Expressed Sequence Tag (EST) database using the program BLASTN (Altschul *et al* 1990) and was found to match numerous human and mouse entries (Table 4 and Figure 2). These matching ESTs were further used to identify overlapping entries in the EST database (Table 5). The nucleotide sequences of these human ESTs were complied using MacVector 4.2.1 software (IBI-Kodak) to produce the cDNA sequence shown in Figure 1. EST entries AA074703 and AA134788 are closely related at the nucleotide level to *mcg4* and it is, therefore, likely that *mcg4* is a member of a newly discovered gene family (Figure 8).

The cDNA sequence of *mcg4* was translated in all possible reading frames and compared to the GenBank non-redundant protein database using the program BLASTX (Altschul *et al*, 1990) at the National Center for Biotechnology Information (http//www.ncbi.nih.gov.nlm). As the protein appeared to be novel, a translation of the longest reading frame for the *mcg4* cDNA was aligned to the EST database using the program TBLASTN, which performed a dynamic translation of the EST database in all 6 frames. The search results indicated that the nematode *C. elegans* had an MCG4-like protein (Figure 3), with the matching domains containing a spatial sequence of Cysteine and Histidine residues which resembled a zinc-finger structure (Figure 4). The program BLASTP was used, therefore, to conduct sensitive searches of the protein databases for similar zinc-finger motifs. A weak match to the putative zinc-finger domain was observed for another protein from *C. elegans* (Figure 5) and a poorer match for the GATA-binding transcription factor from *S. pombe* (Figure 6). The putative initiation codon of human *mcg4* is not preceded by an in-frame stop codon and it is therefore possible that the cDNA described in Figure 1 is a truncated form. However, sequence alignment of human and mouse *mcg4* ESTs showed a lower degree of nucleotide conservation prior to the assigned initiation codon, thus supporting the notion that the region represents the 5′ UTR (Figure 9). To determine the expression pattern of *mcg4*, 15$\mu$g of the total cellular RNA (RNeasy Mini Kit, Qiagen) from various human cell lines grown in culture were electrophoresed through 1.2% w/v MOPS/formaldehyde gels and blotted onto nylon membranes (Amersham) by capillary transfer

using 20 x SSC (Sambrook *et al,* 1989). Filters were subsequently UV-fixed and hybridised overnight at 65°C to a radiolabelled ($^{32}$P-dCTP) cDNA probe (Church and Gilbert, 1984) for *mcg4*. After washes in 0.1 x SSC/0.1% w/v SDS at 65°C for 1 hour, the filters were air-dried and exposed to X-ray film. This Northern analysis showed that *mcg4* is expressed as a 1.6kb message in numerous tissues including breast, ovary, bladder, lung and keratinocytes (Figure 7).

**EXAMPLE 7**

A human gene (designated *mcg7*) was identified and isolated from chromosome 11q13 which encodes a protein that bears striking homology with guanine nucleotide exchange factors (GEFs) from a wide variety of organisms (Fig. 12).

**EXAMPLE 8**

The composite *mcg7* cDNA sequence is at least 2.4kb in length and Figure 13(a) shows a predicted translation product of at least 609 amino acids beginning at methionine 120. An alternative start site due to alternate exon splicing (indicated in lower case) may yield a protein of 671 amino acids starting at methionine 58 (Fig. 13a).

**EXAMPLE 9**

An *mcg7* homologue from *C. elegans* has been identified, the product of which is highly conserved with that of MCG7 (Fig. 14). There are several salient features of the protein which have been underlined in Fig. 14 - namely: a guanine nucleotide binding region, a diacylglycerol binding region, and "EF-hand"-calcium binding regions. In addition, there are several potential cAMP, protein kinase C, and casein kinase II phosphorylation sites, as well as a number of potential sites for glycosylation (not indicated).

**EXAMPLE 10**

A number of partial human and murine EST clones exist for *mcg*7. The GenBank database

contains a cDNA (Acc. no. Y12336) encoding a full-length open reading frame (ORF) for human *mcg7* as well as a partial murine *mcg7* ORF (Y12339). In addition, the complete genomic sequence of the human *mcg7* gene is contained within GenBank entry AC000134.

## EXAMPLE 11

The best characterised GEFs are members of the family of *ras* oncoproteins, which play a pivotal role in signal transduction and when mutated are responsible for tumour development. A variety of therapeutic regimes for cancer treatment have been designed to specifically interfere with the *ras* signalling pathways. There is potential, therefore that the product of *mcg7* could also be a target for such clinical strategies.

## EXAMPLE 12

The nucleotide sequence for *mcg7* cDNA was extended 5' with genomic DNA sequence from Genbank accession number AC000134 (positions 1-321) and analysed for additional coding sequence 5' to the putative initiation codon (nt 681-683) (Fig. 16). An additional in-frame ATG occurs at position nt 495-497 when the alternatively splice exon (position nt 504-609) is present (also shown in Fig. 13(a)). This closely matches the Kozak consensus. When this exon is absent, then the ATG is not in-frame and other possible initiation codons are absent (resulting translation shown in lower case lettering) (also shown in Fig. 13(b)). Further evidence that the initiation codon at position nt 681-683 is the true initiation site is given in Figure 15.

Alignment of human and a partial murine *mcg7* cDNA sequences is shown in Figure 15. The putative initiation codon is at position nt 360-362. Both murine ESTs appear to have an upstream in-frame stop codon at position nt 326-328, downstream of the differentially spliced exon and the sequence alignment thus suggests that this region represents the 5′ UTR of *mcg7*.

Furthermore, similarity with the *C. elegans* homologue strongly suggest that the ATG codon at position nt 360-362 encodes the N-terminus of MCG7.

## EXAMPLE 13

Figure 17 shows data from experiments indicating that a truncated version of MCG7 when expressed as a GST fusion protein (construct B in Fig. 18) can function as a Ras-guanine nucleotide exchange factor. In brief, Ras (unprocessed and as a GST fusion protein) is loaded with $^3$H-GDP then incubated in the presence of excess cold GTP ± GST-MCG7. Full details of this assay can be found in Porfiri *et al.*

## EXAMPLE 14

Nucleotide sequence data generated from cosmid clone cSRL-20h12 with the T7 primer (Promega, and Applied Biosystems Incorporated dye terminator sequencing kit) were aligned to the GenBank Expressed Sequence Tag (EST) database using the program BLASTN (Altschul *et al*, 1990) and was found to match GenBank entries T78563 (clone 113434) TO9103 (clone HIBBP12) and AA035643 (clone 471819). EST clones 113434 and 471819 were obtained from Genome Systems Inc. and these DNAs were sequenced on both strands with gene-specific primers (Table 5) to generate the cDNA sequence of *mcg*7 shown in Figures 13(a) and (b).

The cDNA sequence of *mcg*7 was translated in all possible reading frames and compared to the GenBank non-redundant protein database using the program BLASTX (Altschul *et al*, 1990) and the coding region was assigned on the basis of showing homology to the *C. elegans* protein F25B3.3 (Figure 14). The *mcg*7 cDNA composite was suspected to contain a single nucleotide error that originated from clone 471819 and the correct nucleotide sequence was, therefore, sought by reverse transcription-polymerase chain reaction (RT-PCR) of the cDNA fragment from a human cDNA pool. Total RNA was extracted from a human lymphoblastoid cell line using an RNeasy Mini Kit (Qiagen). cDNA synthesis was conducted with the reverse transcriptase Superscript II RNaseH- (GIBCO, BRL) and random hexamers using the procedure recommended by the manufacturer (GIBCO, BRL). One fortieth of the cDNA mix was subjected to 35 cycles of PCR using the following cycling conditions: 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 90 seconds. The 50$\mu$l reaction mix consisted of 1x reaction buffer (Dade Scientific), 2mM dNTP mix, 20pmol of primers (see Table 6) MCG7UF (within the

variably spliced exon of Figure 13(b), between nucleotide positions 184-201) and SGCADRV2 (between nucleotide positions 866-846 of Figure 13(a)) and 10 units of Dynazyme (Dade Scientific). The resulting PCR product was cloned into the pGEM-T vector (Promega) using standard methodology and sequenced using gene-specific primers. The correct nucleotide sequence of *mcg*7 (as shown in Figure 13(a)) matches that of the recently release GenBank entry Y12336. A partial mouse *mcg*7 cDNA sequence can also be found in GenBank entry Y12339.

## EXAMPLE 15

The coding sequence of *mcg*7 was cloned into vectors for expression in both bacterial and mammalian cells. In addition to the full-length constructs, the deletion constructs shown in Figure 18 were designed to retain the guanine nucleotide exchange (GEF) domain. For prokaryotic expression, the *mcg*7 coding region was inserted downstream of and in-frame with the Sj26 cassette of the pGEX (Pharmacia) series of vectors (Smith and Johnson, 1988) using standard cloning techniques (Sambrook *et al,* 1989). For mammalian expression, the *mcg*7 coding sequence was first *myc*-tagged at the N-terminus and then ligated into the expression vector pc Exv-n using standard cloning techniques. Ligation junctions of the constructs were sequences as the cloning strategies inadvertently changed or introduced additional amino acids as shown below.

**Construct (A):** EST clone 113434 was digested with *Apa*I (Figure 13(a), nucleotide positions 1022 to >2416 (within the vector)), blunt-ended with T4 DNA polymerase according to the specifications of the manufacturer (New England Biolab) and ligated into the *Sma*I site of pGEX-3X.

Sequence of the pGEX and *mcg*7 (underlined) junction:

pGEX-3X *mcg*7 (1022)

Sj26 ... GGG ATC CCC CTG GTC [SEQ ID NO:19]

additional amino acids Gly Ile Pro

**Construct (B):** EST clone 113434 was digested with *Eco*RI (Figure 13(a), nucleotide

positions <695 (within the vector) to 1711) and ligated into the *Eco*RI site of pGEX-1.

Sequence of the pGEX and *mcg*7 (underlined) junction:

```
                pGEX-1              mcg7 (695)
                Sj26 ... GAA TTC GGC ACG AGC CGA CGG [SEQ ID NO:20]
additional amino acids   Glu  Phe  Gly  Thr  Ser
```

**Construct (C):** full-length *mcg*7: The pGEM-T clone containing the 5′ end of the *mcg*7 coding region was digested with *Apa*I (subsequently blunt-ended with T4 DNA polymerase) and *Bst*XI to liberate the fragment between nucleotide positions 336 and 830 of Figure 13(a). Clone 113434 was digested with *Bst*XI and *Hind*III (vector derived) to liberate a fragment between nucleotide positions 830 > and 2416 (vector derived) of Figure 13(a). A pGEM-11zf vector (Promega) containing the *myc*-tag was digested with *Apa*I (subsequently blunt-ended with T4 DNA polymerase) and *Hind*III, and ligated with the 2 inserts described above.

Sequence of the *myc*-tag/*mcg7* junction [SEQ ID NOs:21/22]:

```
----------myc-tag---------------     vector BamHI   mcg7 5' UTR (337)      start
ATGGAGCAGAAGCTGATCTCCGAGGAGGACCTG CCCGGGGCAGCTggatccG CAGCCCACCCCGCGCCGGCGGCCATG
 M  E  Q  K  L  I  S  E  E  D  L   P  G  A  A  G  S   A  A  H  P  A  P  A  A  M
                                  -------------additional amino acids---------
```

The *myc*-tagged full-length *mcg*7 insert in pGEM-11zf was then excised with *Sac*I and *Hind*III (both vector derived) and directionally cloned into the mammalian expression vector pEXV (Beranger *et al*, 1994).

**Construct (D):** Construct (C) in pGEM-11zf was sequentially digested with *Hind*III (this site was subsequently blunt-ended with T4 DNA polymerase) then *Bam*HI, and ligated into pGEX-2T digested with *Bam*HI and *Sma*I. Digestion with *Bam*HI, and ligated into pGEX-2T digested with *Bam*HI and *Sma*I. Digestion with *Bam*HI removed the *myc*-tag of Construct (C).

Sequence of the pGEX and *mcg*7 [SEQ ID NO:23/24] (underlined) junction:

```
pGEX-2    BamHI mcg7 (337)
Sj26  ... gga tcc GCA GCC CAC CCC GCG CCG GCG GCC ATG
          Gly Ser Ala Ala His Pro Ala Pro Ala Ala Met
          -----------additional amino acids------------
```

## EXAMPLE 16

Overnight bacterial cultures containing the pGEX plasmid were used to inoculate 500ml of Luria Broth media containing 50$\mu$g/ml ampicillin. The cultures were grown to an OD of ~0.8 and then induced with 1mM of IPTG for up to 3 hours at 37°C. The bacteria were pelleted and resuspended in 15 ml of STE buffer (10mM Tris pH 8.0, 150 mM NaCl and 1mM EDTA) with 1 mg/ml lysozyme. The mixture was left on ice for more than 1 hour and subsequent steps were performed at 4°C. Protease inhibitors aprotinin, pepstatin and leupeptin were added at final concentrations of 25$\mu$g/ml, prior to the addition of Triton-X-100 (2% v/v final) and n-lauroyl sarcosine (1.5% w/v final). The lysate was sonicated for ~1 minute and pelleted at 14,000 x g for 15 minutes. 100 $\mu$l of 50% w/v glutathione-sephadex bead slurry (in PBS) was added per ml of supernatant. Following a 30 minute incubation at 4°C, the beads were washed three times with NETN (20mM Tris-HC1 pH 8.0, 100mM NaCl, 1mM EDTA, 0.5% NP40), once with NETN-HS (equivalent to NETN but with 1M NaCl), and once in NETN. The bound protein was directly analysed by SDS-polyacrylamide gel electrophoresis (PAGE) as described below or the bound protein was eluted from the beads with the following elution buffer (50mM Tris pH 8.0, 150mM NaCl, 5mM $MgCl_2$, 1mM DTT, 10mM reduced glutathione) for use in GDP release assays.

## EXAMPLE 17

Twenty microlitres of GST-sepharose-bound MCG7 were added to an equal volume of 2 x sample loading dye (100mM Tris pH6.8, 2% v/v mercaptoethanol, 4% w/v SDS, 0.2% w/v bromophenol blue, 20% v/v glycerol), boiled for 5 min and loaded onto a 7.5% w/v SDS-PAGE gel (Sambrook *et al*, 1989). The Coomassie brilliant blue stained gel (Sambrook *et al*, 1989)

typically displayed a protein doublet, running between 87-95 kDa consisting of the MCG7-GST fusion and a slightly smaller, co-purified contaminating *E. coli* protein of ~105kDa. The calculated molecular weight of full-length MCG7 is 77.5 kDa (Construct (D)) and the GST component has a molecular weight of 26kDa, hence, the recombinant protein runs slightly smaller than predicted. A Western blot of the same gel probed with anti-GST antibody yields an MCG7-specific band at the same position as that of the stained gel.

## EXAMPLE 18

Assumptions: (a) GST-Ras molecular weight = 50 kD; (b) Concentration of GST-Ras solution = 1mg/ml = 20$\mu$M; (c) [$^3$H]-GDP is 1mCi/ml and 13.3Ci/mmol, therefore [ H]-GDP concentration = 75 $\mu$M and 1pmol [$^3$H]-GDP=15,466 cpm; (d) Elution buffer = Buffer E = 20 mM Tris-Cl, pH7.5; 50mM NaCl; 5mM $MgCl_2$; 1mM DTT (added just before use). Buffer E + BSA= Buffer E+1mg/ml BSA (added just before use).

Mix together, in the following order and mix well after each addition:
10$\mu$l (=10$\mu$g) GST-Ras (@1mg/ml in Buffer E), 463$\mu$l Buffer E + BSA, 7$\mu$l [$^3$H]-GDP, 10ml 490 $\mu$M EDTA. Incubate @ RT for 10 min. Add 10$\mu$l 0.5 M $MgCl_2$ and mix well. Incubate @ RT for 10 min. Place on ice. During the first incubation the excess EDTA concentration is 5mM, during the second incubation the excess Mg concentration is 5mM. The [$^3$H]-GDP concentration is 1$\mu$M and the final concentration of GST-Ras is 400nM. Thus 20ml of the final mix will contain 8pmol of GST-Ras protein. Specific activity of GDP is 15,446 cpm/pmol x (1/1.4) = 11,047 cpm/pmol.

## EXAMPLE 19

Exchange Ras with labelled GDP as above. Add unlabelled GTP (stock = 100mM, pH7) to 1 mM. Adjust Mg concentration by adding 5$\mu$l 0.5 EDTA to labelled Ras, 5$\mu$l 0.5M EDTA to 500$\mu$l MCG7, and 5$\mu$l 0.5M EDTA to 500$\mu$l Buffer E + BSA. On ice set up microfuge tubes with 40$\mu$l Ras-GDP (in triplicate) with 40$\mu$l MCG7 or Buffer E + BSA (control). Transfer tubes to heat block @ 25°C and incubate for 10, 20 or 30 min. Stop exchange reactions with 1ml of

ice cold buffer E and place on ice. Pre-soak nitrocellulose filters, pore size 45$\mu$m, in Buffer E. Assemble the vacuum manifold apparatus (Millipore) with wet filters and plug the wells with rubber bunds. Switch on the vacuum pump. Remove the first plug, aliquot the sample and once it has been sucked through, wash the filter with 10ml of ice cold Buffer E. Remove next plug etc and continue round the manifold. Take manifold apart. Pin the filters to a pin board reserved for [$^3$H]. Air dry. Take up in 4ml scintillation fluid and count. These studies have been carried out with a truncated MCG7-GST fusion protein (amino acids 341 of Figure 13a to stop encoded within construct B).

## EXAMPLE 20

A human gene was identified from chromosome 11q13 that encodes a new member of the DnaJ family of proteins (designated MCG18). This gene (*mcg18)* is expressed as an ~1.4kb mRNA (Fig. 28) and is predicted to encode a 241 amino acid product (Fig. 19).

## EXAMPLE 21

MCG18 has partial homology to *E. coli* dnaJ and other human DnaJ family members in that it contains the J domain (Fig. 20).

## EXAMPLE 22

MCG18 has greatest homology to functionally undefined proteins from *C. elegans* (Fig. 21) and *S. pombe* (Fig. 22) that also feature the J domain but maintain sequence similarity through the central and C-terminal regions of the proteins.

## EXAMPLE 23

The J domain is proposed to mediate interaction with heat shock protein (Hsp70) 70 and consist of some 70 amino acids, frequently located at the N-terminus of the protein. One of these proteins, tumorous imaginal discs (Tid58) from *Drosophila virilis* (Fig. 23) functions as a

tumour suppressor.

## EXAMPLE 24

A comparison of homology between MCG18 and human DnaJ proteins HDJ-2/H5DJ, HDJ-1/HSP40 and HSJ1 is shown in Fig. 24.

## EXAMPLE 25

During the sequence characterisation of the *VRF/VEGFB* promoter region on cosmid CLGW4 [Grimmond *et al,* 1996], which maps to chromosome 11q13 the inventors identified a sequence that exactly matched numerous human and mouse expressed sequence tags (ESTs) in the EST database from a gene which we designated *mcg18.* EST clones for human (GenBank accession number T69741, clone 108172; accession number H40901, clone 177008) and mouse *mcg18* (accession number W34884, clone 350966; accession number W64183, clone 385535) were obtained from Genome Systems Inc. and sequenced with the gene-specific primers shown in Table 7. The EST clones listed in Table 8 were also utilised in generating the full-length coding sequence for human (Figure 19) and mouse (Figure 25) *mcg18.* The EST database also contained *mcg18* cDNA entries that were alternately (or partially) spliced, and in order to understand their ability to encode new polypeptides, the gene structure of *mcg18* was determined by sequencing human and mouse genomic templates with gene-specific primers.

Genomic fragments containing the human [Grimmond *et al,* 1996] and murine genes [Townson *et al,* 1996] have been previously reported. Cosmid CLGW4 contains the entire human gene and λ121 contains the entire mouse gene, as determined by direct sequencing of the templates with the oligonucleotides listed in Table 7. Plasmids containing sub-fragments of λ121 and cosmid CLGW4 were prepared using plasmid purification kits (Qiagen) and sequenced as described previously [Grimmond *et al,* 1996; Townson *et al,* 1996] using primers designed against cDNA and genomic sequences. The BLAST suite of programs [Altschul *et al,* 1990] was used to compare the sequence data against the nucleotide and protein databases at the National Center for Biotechnology Information (http//www.ncbi.nih.gov.nlm). The sequence

data were compiled using MacVector 4.2.1 software (IBI-Kodak). ClustalW sequence alignments [Thompson *et al*, 1994] were conducted using the Australian National Genome Information Service computer faculty at the University of Sydney, Australia.

The cDNA sequence of human *mcg18* (Figure 19) was translated in all possible reading frames and compared to the GenBank non-redundant protein database using the program BLASTX [Altschul *et al*, 1990] and the coding region was identified on the basis of showing homology to the DnaJ family of proteins (Figure 20). The DnaJ domain is encoded within the longest open reading frame and the assigned initiation codon is preceded by an in-frame stop codon (Figure 27). Similar database search results were obtained for the mouse *mcg18* cDNA, and the alignment of human and mouse protein sequences is shown in Figure 26. MCG18 has greatest homology to gene products from *C. elegans* (Figure 21) and *S. pombe* (Figure 22). Although it shares a similar J-domain, MCG18 does not contain other domains described for the tumour suppressor gene from *D. virilis* (Figure 23), nor is it a homologue of other reported human J-domain-containing proteins (Figure 24).

To determine the expression pattern of *mcg18*, 15$\mu$g of total cellular RNA (RNeasy Mini Kit, Qiagen) from various human cell lines grown in culture were electrophoresed through 1.2% MOPS/formaldehyde gels and blotted onto nylon membranes (Amersham) by capillary transfer using 20 x SSC (Sambrook *et al*, 1986). Filters were subsequently UV-fixed and hybridised overnight at 65°C to a radiolabelled ($^{32}$P-dCTP) cDNA probe (Church and Gilbert, 1984) for *mcg18*. After washes in 0.1 x SSC/0.1% w/v SDS for 65°C for 1 hour, the filters were air-dried and exposed to X-ray film. This Northern analysis showed that *mcg18* is expressed as a 1.4kb message in numerous tissues including breast, ovary, bladder, lung and keratinocytes (Figure 28).

# TABLE 4

## ESTs matching *mcg4*

| accession number | seq. run | organism | score | E value | N |
|---|---|---|---|---|---|
| gb\|AA399110\|AA399110 | zt89e06.s1 | Soares testis NHT Homo sa... | 1136 | 4.0e-168 | 2 |
| gb\|N39612\|N39612 | yy51g06.s1 | Homo sapiens cDNA clone 2... | 1521 | 5.3e-168 | 4 |
| gb\|AA514406\|AA514406 | nf57d01.s1 | NCI_CGAP_Co3 Homo sapiens... | 931 | 5.5e-166 | 3 |
| gb\|AA544946\|AA544946 | vk38e02.r1 | Soares mouse mammary glan... | 1207 | 8.4e-164 | 2 |
| gb\|AA450076\|AA450076 | zx42a04.s1 | Soares total fetus Nb2HF8... | 691 | 2.3e-160 | 4 |
| gb\|AA535731\|AA535731 | nf88f07.s1 | NCI_CGAP_Co3 Homo sapiens... | 796 | 3.5e-158 | 4 |
| gb\|W79710\|W79710 | zd86f01.r1 | Soares fetal heart NbHH19... | 1644 | 1.1e-157 | 4 |
| gb\|AA503531\|AA503531 | ne47e08.s1 | NCI_CGAP_Co3 Homo sapiens... | 736 | 4.0e-156 | 4 |
| gb\|AA450132\|AA450132 | zx42a04.r1 | Soares total fetus Nb2HF8... | 1955 | 3.9e-155 | 1 |
| gb\|AA398068\|AA398068 | zt89f06.r1 | Soares testis NHT Homo sa... | 1315 | 5.4e-148 | 2 |
| gb\|W60405\|W60405 | zd29h08.r1 | Soares fetal heart NbHH19... | 1022 | 1.8e-139 | 4 |
| gb\|W81382\|W81382 | zd86f01.s1 | Soares fetal heart NbHH19... | 605 | 3.5e-125 | 5 |
| gb\|AA047617\|AA047617 | zf13f07.s1 | Soares fetal heart NbHH19... | 922 | 4.6e-125 | 2 |
| gb\|AA282175\|AA282175 | zt02d03.s1 | NCI_CGAP_GCB1 Homo sapien... | 1577 | 2.0e-123 | 1 |
| gb\|AA242159\|AA242159 | my30d04.r1 | Barstead mouse pooled org... | 866 | 7.7e-117 | 2 |
| gb\|AA068680\|AA068680 | mm61a05.r1 | Stratagene mouse embryoni... | 1280 | 1.6e-98 | 1 |
| gb\|W46766\|W46766 | zc36b07.s1 | Soares senescent fibrobla... | 506 | 9.6e-92 | 3 |
| gb\|N93704\|N93704 | zb51c04.s1 | Soares fetal lung NbHL19W... | 584 | 9.0e-91 | 4 |
| gb\|AA155210\|AA155210 | mr98e01.r1 | Stratagene mouse embryoni... | 840 | 7.6e-87 | 2 |
| gb\|AA366022\|AA366022 | EST76915 | Pineal gland II Homo sapien... | 1077 | 2.4e-81 | 1 |
| gb\|AA037691\|AA037691 | zk34h12.s1 | Soares pregnant uterus Nb... | 949 | 2.1e-80 | 2 |
| gb\|W35374\|W35374 | zc07h03.s1 | Soares parathyroid tumor ... | 1016 | 3.1e-76 | 1 |
| dbj\|C00696\|C00696 | HUMGS0008251, | Human Gene Signature, ... | 1009 | 1.2e-75 | 1 |
| gb\|T98249\|T98249 | ye59a07.s1 | Homo sapiens cDNA clone 1... | 998 | 6.7e-75 | 1 |
| gb\|W21588\|W21588 | zb51c04.r1 | Soares fetal lung NbHL19W... | 484 | 1.1e-69 | 4 |
| gb\|H32171\|H32171 | EST107015 | Rattus sp. cDNA 5' end. | 828 | 1.1e-60 | 1 |
| gb\|AA108092\|AA108092 | mm89e06.r1 | Stratagene mouse embryoni... | 782 | 1.3e-60 | 2 |
| gb\|AA017857\|AA017857 | mh44d10.r1 | Soares mouse placenta 4Nb... | 665 | 2.5e-60 | 2 |
| gb\|AA037690\|AA037690 | zk34h12.r1 | Soares pregnant uterus Nb... | 540 | 9.4e-53 | 2 |
| gb\|AA531006\|AA531006 | nj07b11.s1 | NCI_CGAP_Pr22 Homo sapien... | 535 | 5.4e-48 | 2 |
| gb\|N46760\|N46760 | yy51g06.r1 | Homo sapiens cDNA clone 2... | 665 | 9.5e-47 | 1 |
| gb\|W23584\|W23584 | zc71d03.s1 | Soares fetal heart NbHH19... | 457 | 1.8e-44 | 2 |
| gb\|W42214\|W42214 | mc69h09.r1 | Soares mouse embryo NbME1... | 460 | 1.3e-38 | 3 |
| gb\|AA244877\|AA244877 | mx25a04.r1 | Soares mouse NML Mus musc... | 429 | 2.9e-25 | 1 |
| gb\|W32939\|W32939 | zc07h03.r1 | Soares parathyroid tumor ... | 320 | 4.8e-18 | 1 |

# TABLE 5

## ESTs matching AA074703 (*mcg4*-related cDNA)

Database: Non-redundant Database of GenBank EST Division
1,222,625 sequences; 449,352,662 total letters.

| Sequences producing High-scoring Segment Pairs: | | | High Score | Smallest Sum Probability P(N) | N |
|---|---|---|---|---|---|
| accession number | seq. run | organism | score | E value | N |
| gb\|AA074703\|AA074703 | zm76g07.r1 | Stratagene neuroepitheli... | 2071 | 4.0e-167 | 1 |
| gb\|AA068680\|AA068680 | mm61a05.r1 | Stratagene mouse embryon... | 1270 | 4.4e-145 | 4 |
| gb\|AA134788\|AA134788 | zm81g02.r1 | Stratagene neuroepitheli... | 946 | 1.3e-144 | 5 |
| gb\|AA399110\|AA399110 | zt89e06.s1 | Soares testis NHT Homo s... | 520 | 8.7e-119 | 6 |
| gb\|N39612\|N39612 | yy51g06.s1 | Homo sapiens cDNA clone ... | 582 | 9.6e-110 | 7 |
| gb\|AA282175\|AA282175 | zt02d03.s1 | NCI_CGAP_GCB1 Homo sapie... | 771 | 9.4e-80 | 3 |
| gb\|W81382\|W81382 | zd86f01.s1 | Soares fetal heart NbHH1... | 329 | 1.6e-75 | 6 |
| gb\|AA544946\|AA544946 | vk38e02.r1 | Soares mouse mammary gla... | 644 | 9.6e-63 | 2 |
| gb\|W35374\|W35374 | zc07h03.s1 | Soares parathyroid tumor... | 294 | 4.5e-42 | 4 |
| gb\|W57106\|W57106 | md57c12.r1 | Soares mouse embryo NbME... | 394 | 1.9e-30 | 2 |
| gb\|AA244877\|AA244877 | mx25a04.r1 | Soares mouse NML Mus mus... | 162 | 2.1e-27 | 4 |
| gb\|AA017857\|AA017857 | mh44d10.r1 | Soares mouse placenta 4N... | 230 | 3.7e-23 | 3 |
| gb\|AA531006\|AA531006 | nj07b11.s1 | NCI_CGAP_Pr22 Homo sapie... | 139 | 2.3e-19 | 3 |
| gb\|H32171\|H32171 | EST107015 | Rattus sp. cDNA 5' end. | 207 | 2.6e-10 | 2 |
| gb\|W79710\|W79710 | zd86f01.r1 | Soares fetal heart NbHH1... | 157 | 0.0073 | 1 |

**TABLE 6**

***mcg7*-specific oligonucleotides**

| name | sequence (5′ to 3′) | SEQ ID NOs. |
|---|---|---|
| M1044R | GGA CAA AGT GTG TGA TGA ACC | SEQ ID NO:25 |
| MCG7-GEF-REV2 | CTC ATC CTC CGT CTG ATA CTG | SEQ ID NO:26 |
| M7R | GTA GAT GTG GAT CAG CTT GG | SEQ ID NO:27 |
| MCG7 CA FOR | AGG TGG AGA ATG GTC AAGG | SEQ ID NO:28 |
| MCG7-GEF-REV | GTC ATA GTC TGT CTC CTA CT | SEQ ID NO:29 |
| MCG7 GEF FOR | ACA TAG ACA GCG TGC CTA CC | SEQ ID NO:30 |
| MCG7-PKC-REV | TAC AAC CTT AGG GAC ACC AG | SEQ ID NO:31 |
| MCG7-PKC-FOR | TGC TGA GCC TGC TCA CGG TG | SEQ ID NO:32 |
| T09103F | CAA GTG AAC AGC ACG TCC | SEQ ID NO:33 |
| M7F | GAC TAT CTC AAG GAC CAG CTG | SEQ ID NO:34 |
| MCG7UF | GGT TCG GTC CGA GCC CGG | SEQ ID NO:35 |
| SGCADRV2 | GGA GCG ATA CTC CAA GTA GGT | SEQ ID NO:36 |

**TABLE 7**

***mcg18*-SPECIFIC OLIGONUCLEOTIDES**

| name | sequence 5′ to 3′ |
|---|---|
| HVESTF | AGC GGG CCA GGC CCC TTC [SEQ ID NO:37] |
| HV195F | CAT CCT GGT CCA ATG CGC TC [SEQ ID NO:38] |
| HV387F2 | GCA CTG AGG AAG TTA AAC GAG C [SEQ ID NO:39] |
| HV408R | GCT CGT TTA ACT TCC TCA GTG C [SEQ ID NO:40] |
| EXON1REV | GCT CAG CTC CAC AAA GCG GCT [SEQ ID NO:41] |
| HVEST426F | ACC AGC TCC GCT CAG GTA G [SEQ ID NO:42] |
| HVEST623R | TCC AGG AGC TGT GTG TTT GG [SEQ ID NO:43] |
| SGVESTF3 | CCA GTT TCA CAG CGT GAG G [SEQ ID NO:44] |
| HVEST631R | CAG CAT GAG GAG GAG GCA G [SEQ ID NO:45] |

## TABLE 8

## EST CLONE SEQUENCES USED TO GENERATE HUMAN AND MOUSE *mcg18* cDNA SEQUENCE COMPOSITES

| EST clone number | organism | GenBank accession number |
|---|---|---|
| 1g2815 | human | D45683 |
| 001-T2-18 | human | F17225 |
| 273748 | human | N37043 |
| 177008 | human | H40901 and H40939 |
| 258011 | human | N30776 |
| 276887 | human | N44004 |
| 108172 | human | T69741 |
| 307529 | human | W21083 and W32579 |
| 342027 | human | W60283 |
| 354288 | mouse | W44038 |
| 350966 | mouse | W348844 |
| 426261 | mouse | AA002868 |
| 368185 | mouse | W53911 |
| 385535 | mouse | W64183 |
| 404472 | mouse | W82959 |
| 406437 | mouse | W83482 |

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: (OTHER THAN US): The Council of The Queensland Institute of Medical Research
(US ONLY): HAYWARD Nicholas, SILINS Ginters, GRIMMOND Sean, GARTSIDE Michael and HANCOCK, John

(ii) TITLE OF INVENTION:A NOVEL GENE AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 45

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 22-MAY-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PO6973
(B) FILING DATE: 23-MAY-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PO6974
(B) FILING DATE: 23-MAY-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PO6972
(B) FILING DATE: 23-MAY-1997

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PP1459
(B) FILING DATE: 22-JAN-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PP1460
(B) FILING DATE: 22-JAN-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PP1458
(B) FILING DATE: 22-JAN-1998
(C) CLASSIFICATION:

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(C) TELEX: AA 31787

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Cys Xaa Xaa Cys Xaa Gly Xaa Gly
                 5
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1242 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 30..959

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
TCAGTAAACA CAGAGACTGG GGATCGATC ATG GGG CTT TGT AAG TGC CCC AAG       53
                                Met Gly Leu Cys Lys Cys Pro Lys
                                 1               5

AGA AAG GTG ACC AAC CTG TTC TGC TTC GAA CAT CGG GTC AAC GTC TGC      101
Arg Lys Val Thr Asn Leu Phe Cys Phe Glu His Arg Val Asn Val Cys
     10                  15                  20

GAG CAC TGC CTG GTA GCC AAT CAC GCC AAG TGC ATC GTC CAG TCC TAC      149
Glu His Cys Leu Val Ala Asn His Ala Lys Cys Ile Val Gln Ser Tyr
 25                  30                  35                  40

CTG CAA TGG CTC CAA GAT AGC GAC TAC AAC CCC AAT TGC CGC CTG TGC      197
Leu Gln Trp Leu Gln Asp Ser Asp Tyr Asn Pro Asn Cys Arg Leu Cys
                 45                  50                  55

AAC ATA CCC CTG GCC AGC CGA GAG ACG ACC CGC CTT GTC TGC TAT GAT      245
Asn Ile Pro Leu Ala Ser Arg Glu Thr Thr Arg Leu Val Cys Tyr Asp
             60                  65                  70

CTC TTT CAC TGG GCC TGC CTC AAT GAA CGT GCT GCC CAG CTA CCC CGA      293
Leu Phe His Trp Ala Cys Leu Asn Glu Arg Ala Ala Gln Leu Pro Arg
         75                  80                  85

AAC ACG GCA CCT GCC GGC TAT CAG TGC CCC AGC TGC AAT GGC CCC ATC      341
Asn Thr Ala Pro Ala Gly Tyr Gln Cys Pro Ser Cys Asn Gly Pro Ile
     90                  95                 100

TTC CCC CCA ACC AAC CTG GCT GGC CCC GTG GCC TCC GCA CTG AGA GAG      389
Phe Pro Pro Thr Asn Leu Ala Gly Pro Val Ala Ser Ala Leu Arg Glu
105                 110                 115                 120
```

```
AAG CTG GCC ACA GTC AAC TGG GCC CGG GCA GGA CTG GGC CTC CCT CTG      437
Lys Leu Ala Thr Val Asn Trp Ala Arg Ala Gly Leu Gly Leu Pro Leu
                125                 130                 135

ATC GAT GAG GTG GTG AGC CCA GAG CCC GAG CCC CTC AAC ACG TCT GAC      485
Ile Asp Glu Val Val Ser Pro Glu Pro Glu Pro Leu Asn Thr Ser Asp
            140                 145                 150

TTC TCT GAC TGG TCT AGT TTT AAT GCC AGC AGT ACC CCT GGA CCA GAG      533
Phe Ser Asp Trp Ser Ser Phe Asn Ala Ser Ser Thr Pro Gly Pro Glu
        155                 160                 165

GAG GTA GAC AGC GCC TCT GCT GCC CCA GCC TTC TAC AGC CGA GCC CCC      581
Glu Val Asp Ser Ala Ser Ala Ala Pro Ala Phe Tyr Ser Arg Ala Pro
    170                 175                 180

CGG CCC CCA GCT TCC CCA GGC CGG CCC GAG CAG CAC ACA GTG ATC CAC      629
Arg Pro Pro Ala Ser Pro Gly Arg Pro Glu Gln His Thr Val Ile His
185                 190                 195                 200

ATG GGC AAT CCT GAG CCC TTG ACT CAC GCC CCT AGG AAG GTG TAT GAT      677
Met Gly Asn Pro Glu Pro Leu Thr His Ala Pro Arg Lys Val Tyr Asp
                205                 210                 215

ACG CGG GAT GAT GAC CGG ACA CCA GGC CTC CAT GGA GAC TGT GAC GAT      725
Thr Arg Asp Asp Asp Arg Thr Pro Gly Leu His Gly Asp Cys Asp Asp
            220                 225                 230

GAC AAG TAC CGA CGT CGG CCG GCC TTG GGT TGG CTG GCC CGG CTG CTA      773
Asp Lys Tyr Arg Arg Arg Pro Ala Leu Gly Trp Leu Ala Arg Leu Leu
        235                 240                 245

AGG AGC CGG GCT GGG TCT CGG AAG CGG CCG CTG ACC CTG CTC CAG CGG      821
Arg Ser Arg Ala Gly Ser Arg Lys Arg Pro Leu Thr Leu Leu Gln Arg
    250                 255                 260

GCG GGG CTG CTG CTA CTC TTG GGA CTG CTG GGC TTC CTG GCC CTC CTT      869
Ala Gly Leu Leu Leu Leu Leu Gly Leu Leu Gly Phe Leu Ala Leu Leu
265                 270                 275                 280

GCC CTC ATG TCT CGC CTA GGC CGG GCC GCA GCT GAC AGC GAT CCC AAC      917
Ala Leu Met Ser Arg Leu Gly Arg Ala Ala Ala Asp Ser Asp Pro Asn
                285                 290                 295

CTG GAC CCA CTC ATG AAC CCT CAC ATC CGC GTG GGC CCC TCC TGA          962
Leu Asp Pro Leu Met Asn Pro His Ile Arg Val Gly Pro Ser  *
            300                 305                 310

GCCCCCTTGC TTGTGGCTAG GCCAGCCTAG GATGTGGGTT CTGTGGAGGA GAGGCGGGGT   1022

AATGGGGAGG CTGAGGGCAC CTCTTCACTG CCCCTCTCCC TCAAGCCTAA GACACTAAGA   1082

CCCCAGACCC AAAGCCAAGT CCACCAGAGT GGCTCGCAGG CCAGGCCTGG AGTCCCCGTG   1142

GGTCAAGCAT TTGTCTTGAC TTGCTTTCTC CCGGGTCTCC AGCCTCCGAC CCCTCGCCCC   1202

ATGAAGGAGC TGGCAGGTGG AAATAAACAA CAACTTTATT                         1242
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 310 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gly Leu Cys Lys Cys Pro Lys Arg Lys Val Thr Asn Leu Phe Cys
 1               5                  10                  15

Phe Glu His Arg Val Asn Val Cys Glu His Cys Leu Val Ala Asn His
            20                  25                  30

Ala Lys Cys Ile Val Gln Ser Tyr Leu Gln Trp Leu Gln Asp Ser Asp
        35                  40                  45

Tyr Asn Pro Asn Cys Arg Leu Cys Asn Ile Pro Leu Ala Ser Arg Glu
    50                  55                  60

Thr Thr Arg Leu Val Cys Tyr Asp Leu Phe His Trp Ala Cys Leu Asn
65                  70                  75                  80

Glu Arg Ala Ala Gln Leu Pro Arg Asn Thr Ala Pro Ala Gly Tyr Gln
                85                  90                  95

Cys Pro Ser Cys Asn Gly Pro Ile Phe Pro Pro Thr Asn Leu Ala Gly
            100                 105                 110

Pro Val Ala Ser Ala Leu Arg Glu Lys Leu Ala Thr Val Asn Trp Ala
        115                 120                 125

Arg Ala Gly Leu Gly Leu Pro Leu Ile Asp Glu Val Val Ser Pro Glu
    130                 135                 140

Pro Glu Pro Leu Asn Thr Ser Asp Phe Ser Asp Trp Ser Ser Phe Asn
145                 150                 155                 160

Ala Ser Ser Thr Pro Gly Pro Glu Glu Val Asp Ser Ala Ser Ala Ala
                165                 170                 175

Pro Ala Phe Tyr Ser Arg Ala Pro Arg Pro Pro Ala Ser Pro Gly Arg
            180                 185                 190

Pro Glu Gln His Thr Val Ile His Met Gly Asn Pro Glu Pro Leu Thr
        195                 200                 205

His Ala Pro Arg Lys Val Tyr Asp Thr Arg Asp Asp Asp Arg Thr Pro
    210                 215                 220

Gly Leu His Gly Asp Cys Asp Asp Asp Lys Tyr Arg Arg Arg Pro Ala
225                 230                 235                 240

Leu Gly Trp Leu Ala Arg Leu Leu Arg Ser Arg Ala Gly Ser Arg Lys
                245                 250                 255

Arg Pro Leu Thr Leu Leu Gln Arg Ala Gly Leu Leu Leu Leu Leu Gly
            260                 265                 270

Leu Leu Gly Phe Leu Ala Leu Leu Ala Leu Met Ser Arg Leu Gly Arg
        275                 280                 285

Ala Ala Ala Asp Ser Asp Pro Asn Leu Asp Pro Leu Met Asn Pro His
    290                 295                 300

Ile Arg Val Gly Pro Ser
305                 310
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2415 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..2188

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
CG ATT TCA TTC CTC GCT CCC CAC AGG TCC CTC TCC CCA AAA TAT TCC      47
   Ile Ser Phe Leu Ala Pro His Arg Ser Leu Ser Pro Lys Tyr Ser
     1               5                  10                  15

CAT CTT GTC CTA GCC CAT CCC CCA GAC TAT CTC AAG GAC CAG CTG TCC      95
His Leu Val Leu Ala His Pro Pro Asp Tyr Leu Lys Asp Gln Leu Ser
                 20                  25                  30

CCA CGC CCC CGA CCT CCA CTA GGC CTG TGC CAC CCG CTG CCT GCA GGA     143
Pro Arg Pro Arg Pro Pro Leu Gly Leu Cys His Pro Leu Pro Ala Gly
             35                  40                  45

AGA CGC CCG GTC CCG GGC CGG GTT AGC CCC ATG GGA ACG CAG CGC CTG     191
Arg Arg Pro Val Pro Gly Arg Val Ser Pro Met Gly Thr Gln Arg Leu
         50                  55                  60

TGT GGC CGC GGG ACT CAA GGC TGG CCT GGC TCA AGT GAA CAG CAC GTC     239
Cys Gly Arg Gly Thr Gln Gly Trp Pro Gly Ser Ser Glu Gln His Val
     65                  70                  75

CAG GAG GCG ACC TCG TCC GCG GGT TTG CAT TCT GGG GTG GAC GAG CTG     287
Gln Glu Ala Thr Ser Ser Ala Gly Leu His Ser Gly Val Asp Glu Leu
 80                  85                  90                  95

GGG GTT CGG TCC GAG CCC GGT GGG AGG CTC CCG GAG CGC AGC CTG GGC     335
Gly Val Arg Ser Glu Pro Gly Gly Arg Leu Pro Glu Arg Ser Leu Gly
                100                 105                 110

CCA GCC CAC CCC GCG CCG GCG GCC ATG GCA GGC ACC CTG GAC CTG GAC     383
Pro Ala His Pro Ala Pro Ala Ala Met Ala Gly Thr Leu Asp Leu Asp
            115                 120                 125

AAG GGC TGC ACG GTG GAG GAG CTG CTC CGC GGG TGC ATC GAA GCC TTC     431
Lys Gly Cys Thr Val Glu Glu Leu Leu Arg Gly Cys Ile Glu Ala Phe
        130                 135                 140

GAT GAC TCC GGG AAG GTG CGG GAC CCG CAG CTG GTG CGC ATG TTC CTC     479
Asp Asp Ser Gly Lys Val Arg Asp Pro Gln Leu Val Arg Met Phe Leu
    145                 150                 155

ATG ATG CAC CCC TGG TAC ATC CCC TCC TCT CAG CTG GCG GCC AAG CTG     527
Met Met His Pro Trp Tyr Ile Pro Ser Ser Gln Leu Ala Ala Lys Leu
160                 165                 170                 175

CTC CAC ATC TAC CAA CAA TCC CGG AAG GAC AAC TCC AAT TCC CTG CAG     575
Leu His Ile Tyr Gln Gln Ser Arg Lys Asp Asn Ser Asn Ser Leu Gln
                180                 185                 190

GTG AAA ACG TGC CAC CTG GTC AGG TAC TGG ATC TCC GCC TTC CCA GCG     623
Val Lys Thr Cys His Leu Val Arg Tyr Trp Ile Ser Ala Phe Pro Ala
            195                 200                 205
```

```
GAG TTT GAC TTG AAC CCG GAG TTG GCT GAG CAG ATC AAG GAG CTG AAG        671
Glu Phe Asp Leu Asn Pro Glu Leu Ala Glu Gln Ile Lys Glu Leu Lys
        210                 215                 220

GCT CTG CTA GAC CAA GAA GGG AAC CGA CGG CAC AGC AGC CTA ATC GAC        719
Ala Leu Leu Asp Gln Glu Gly Asn Arg Arg His Ser Ser Leu Ile Asp
    225                 230                 235

ATA GAC AGC GTC CCT ACC TAC AAG TGG AAG CGG CAG GTG ACT CAG CGG        767
Ile Asp Ser Val Pro Thr Tyr Lys Trp Lys Arg Gln Val Thr Gln Arg
240                 245                 250                 255

AAC CCT GTG GGA CAG AAA AAG CGC AAG ATG TCC CTG TTG TTT GAC CAC        815
Asn Pro Val Gly Gln Lys Lys Arg Lys Met Ser Leu Leu Phe Asp His
                260                 265                 270

CTG GAG CCC ATG GAG CTG GCG GAG CAT CTC ACC TAC TTG GAG TAT CGC        863
Leu Glu Pro Met Glu Leu Ala Glu His Leu Thr Tyr Leu Glu Tyr Arg
            275                 280                 285

TCC TTC TGC AAG ATC CTG TTT CAG GAC TAT CAC AGT TTC GTG ACT CAT        911
Ser Phe Cys Lys Ile Leu Phe Gln Asp Tyr His Ser Phe Val Thr His
        290                 295                 300

GGC TGC ACT GTG GAC AAC CCC GTC CTG GAG CGG TTC ATC TCC CTC TTC        959
Gly Cys Thr Val Asp Asn Pro Val Leu Glu Arg Phe Ile Ser Leu Phe
    305                 310                 315

AAC AGC GTC TCA CAG TGG GTG CAG CTC ATG ATC CTC AGC AAA CCC ACA       1007
Asn Ser Val Ser Gln Trp Val Gln Leu Met Ile Leu Ser Lys Pro Thr
320                 325                 330                 335

GCC CCG CAG CGG GCC CTG GTC ATC ACA CAC TTT GTC CAC GTG GCG GAG       1055
Ala Pro Gln Arg Ala Leu Val Ile Thr His Phe Val His Val Ala Glu
                340                 345                 350

AAG CTG CTA CAG CTG CAG AAC TTC AAC ACG CTG ATG GCA GTG GTC GGG       1103
Lys Leu Leu Gln Leu Gln Asn Phe Asn Thr Leu Met Ala Val Val Gly
            355                 360                 365

GGC CTG AGC CAC AGC TCC ATC TCC CGC CTC AAG GAG ACC CAC AGC CAC       1151
Gly Leu Ser His Ser Ser Ile Ser Arg Leu Lys Glu Thr His Ser His
        370                 375                 380

GTT AGC CCT GAG ACC ATC AAG CTC TGG GAG GGT CTC ACG GAA CTA GTG       1199
Val Ser Pro Glu Thr Ile Lys Leu Trp Glu Gly Leu Thr Glu Leu Val
    385                 390                 395

ACG GCG ACA GGC AAC TAT GGC AAC TAC CGG CGT CGG CTG GCA GCC TGT       1247
Thr Ala Thr Gly Asn Tyr Gly Asn Tyr Arg Arg Arg Leu Ala Ala Cys
400                 405                 410                 415

GTG GGC TTC CGC TTC CCG ATC CTG GGT GTG CAC CTC AAG GAC CTG GTG       1295
Val Gly Phe Arg Phe Pro Ile Leu Gly Val His Leu Lys Asp Leu Val
                420                 425                 430

GCC CTG CAG CTG GCA CTG CCT GAC TGG CTG GAC CCA GCC CGG ACC CGG       1343
Ala Leu Gln Leu Ala Leu Pro Asp Trp Leu Asp Pro Ala Arg Thr Arg
            435                 440                 445

CTC AAC GGG GCC AAG ATG AAG CAG CTC TTT AGC ATC CTG GAG GAG CTG       1391
Leu Asn Gly Ala Lys Met Lys Gln Leu Phe Ser Ile Leu Glu Glu Leu
        450                 455                 460

GCC ATG GTG ACC AGC CTG CGG CCA CCA GTA CAG GCC AAC CCC GAC CTG       1439
Ala Met Val Thr Ser Leu Arg Pro Pro Val Gln Ala Asn Pro Asp Leu
    465                 470                 475
```

CTG AGC CTG CTC ACG GTG TCT CTG GAT CAG TAT CAG ACG GAG GAT GAG 1487
Leu Ser Leu Leu Thr Val Ser Leu Asp Gln Tyr Gln Thr Glu Asp Glu
480 485 490 495

CTG TAC CAG CTG TCC CTG CAG CGG GAG CCG CGC TCC AAG TCC TCG CCA 1535
Leu Tyr Gln Leu Ser Leu Gln Arg Glu Pro Arg Ser Lys Ser Ser Pro
500 505 510

ACC AGC CCC ACG AGT TGC ACC CCA CCA CCC CGG CCC CCG GTA CTG GAG 1583
Thr Ser Pro Thr Ser Cys Thr Pro Pro Pro Arg Pro Pro Val Leu Glu
515 520 525

GAG TGG ACC TCG GCT GCC AAA CCC AAG CTG GAT CAG GCC CTC GTG GTG 1631
Glu Trp Thr Ser Ala Ala Lys Pro Lys Leu Asp Gln Ala Leu Val Val
530 535 540

GAG CAC ATC GAG AAG ATG GTG GAG TCT GTG TTC CGG AAC TTT GAC GTC 1679
Glu His Ile Glu Lys Met Val Glu Ser Val Phe Arg Asn Phe Asp Val
545 550 555

GAT GGG GAT GGC CAC ATC TCA CAG GAA GAA TTC CAG ATC ATC CGT GGG 1727
Asp Gly Asp Gly His Ile Ser Gln Glu Glu Phe Gln Ile Ile Arg Gly
560 565 570 575

AAC TTC CCT TAC CTC AGC GCC TTT GGG GAC CTC GAC CAG AAC CAG GAT 1775
Asn Phe Pro Tyr Leu Ser Ala Phe Gly Asp Leu Asp Gln Asn Gln Asp
580 585 590

GGC TGC ATC AGC AGG GAG GAG ATG GTT TCC TAT TTC CTG CGC TCC AGC 1823
Gly Cys Ile Ser Arg Glu Glu Met Val Ser Tyr Phe Leu Arg Ser Ser
595 600 605

TCT GTG TTG GGG GGG CGC ATG GGC TTC GTA CAC AAC TTC CAG GAG AGC 1871
Ser Val Leu Gly Gly Arg Met Gly Phe Val His Asn Phe Gln Glu Ser
610 615 620

AAC TCC TTG CGC CCC GTC GCC TGC CGC CAC TGC AAA GCC CTG ATC CTG 1919
Asn Ser Leu Arg Pro Val Ala Cys Arg His Cys Lys Ala Leu Ile Leu
625 630 635

GGC ATC TAC AAG CAG GGC CTC AAA TGC CGA GCC TGT GGA GTG AAC TGC 1967
Gly Ile Tyr Lys Gln Gly Leu Lys Cys Arg Ala Cys Gly Val Asn Cys
640 645 650 655

CAC AAG CAG TGC AAG GAT CGC CTG TCA GTT GAG TGT CGG CGC AGG GCC 2015
His Lys Gln Cys Lys Asp Arg Leu Ser Val Glu Cys Arg Arg Arg Ala
660 665 670

CAG AGT GTG AGC CTG GAG GGG TCT GCA CCC TCA CCC TCA CCC ATG CAC 2063
Gln Ser Val Ser Leu Glu Gly Ser Ala Pro Ser Pro Ser Pro Met His
675 680 685

AGC CAC CAT CAC CGC GCC TTC AGC TTC TCT CTG CCC CGC CCT GGC AGG 2111
Ser His His His Arg Ala Phe Ser Phe Ser Leu Pro Arg Pro Gly Arg
690 695 700

CGA GGC TCC AGG CCT CCA GAG ATC CGT GAG GAG GAG GTA CAG ACG GTG 2159
Arg Gly Ser Arg Pro Pro Glu Ile Arg Glu Glu Glu Val Gln Thr Val
705 710 715

GAG GAT GGG GTG TTT GAC ATC CAC TTG TA ATAGATGCTG TGGTTGGATC 2208
Glu Asp Gly Val Phe Asp Ile His Leu
720 725

AAGGACTCAT TCCTGCCTTG GAGAAAATAC TTCAACCAGA GCAGGGAGCC TGGGGGTGTC 2268

GGGGCAGGAG GCTGGGGATG GGGGTGGGAT ATGAGGGTGG CATGCAGCTG AGGGCAGGGC 2328

CAGGGCTGGT GTCCCTAAGG TTGTACAGAC TCTTGTGAAT ATTTGTATTT TCCAGATGGA 2388

ATAAAAAGGC CCGTGTAATT AACCTTC 2415

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 728 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Ile Ser Phe Leu Ala Pro His Arg Ser Leu Ser Pro Lys Tyr Ser His
  1               5                  10                  15

Leu Val Leu Ala His Pro Pro Asp Tyr Leu Lys Asp Gln Leu Ser Pro
             20                  25                  30

Arg Pro Arg Pro Pro Leu Gly Leu Cys His Pro Leu Pro Ala Gly Arg
         35                  40                  45

Arg Pro Val Pro Gly Arg Val Ser Pro Met Gly Thr Gln Arg Leu Cys
     50                  55                  60

Gly Arg Gly Thr Gln Gly Trp Pro Gly Ser Ser Glu Gln His Val Gln
 65                  70                  75                  80

Glu Ala Thr Ser Ser Ala Gly Leu His Ser Gly Val Asp Glu Leu Gly
                 85                  90                  95

Val Arg Ser Glu Pro Gly Gly Arg Leu Pro Glu Arg Ser Leu Gly Pro
            100                 105                 110

Ala His Pro Ala Pro Ala Ala Met Ala Gly Thr Leu Asp Leu Asp Lys
        115                 120                 125

Gly Cys Thr Val Glu Glu Leu Leu Arg Gly Cys Ile Glu Ala Phe Asp
    130                 135                 140

Asp Ser Gly Lys Val Arg Asp Pro Gln Leu Val Arg Met Phe Leu Met
145                 150                 155                 160

Met His Pro Trp Tyr Ile Pro Ser Ser Gln Leu Ala Ala Lys Leu Leu
                165                 170                 175

His Ile Tyr Gln Gln Ser Arg Lys Asp Asn Ser Asn Ser Leu Gln Val
            180                 185                 190

Lys Thr Cys His Leu Val Arg Tyr Trp Ile Ser Ala Phe Pro Ala Glu
        195                 200                 205

Phe Asp Leu Asn Pro Glu Leu Ala Glu Gln Ile Lys Glu Leu Lys Ala
    210                 215                 220

Leu Leu Asp Gln Glu Gly Asn Arg Arg His Ser Ser Leu Ile Asp Ile
225                 230                 235                 240

Asp Ser Val Pro Thr Tyr Lys Trp Lys Arg Gln Val Thr Gln Arg Asn
                245                 250                 255

Pro Val Gly Gln Lys Lys Arg Lys Met Ser Leu Leu Phe Asp His Leu
            260                 265                 270
```

Glu Pro Met Glu Leu Ala Glu His Leu Thr Tyr Leu Glu Tyr Arg Ser
        275                 280                 285

Phe Cys Lys Ile Leu Phe Gln Asp Tyr His Ser Phe Val Thr His Gly
    290                 295                 300

Cys Thr Val Asp Asn Pro Val Leu Glu Arg Phe Ile Ser Leu Phe Asn
305                 310                 315                 320

Ser Val Ser Gln Trp Val Gln Leu Met Ile Leu Ser Lys Pro Thr Ala
                325                 330                 335

Pro Gln Arg Ala Leu Val Ile Thr His Phe Val His Val Ala Glu Lys
            340                 345                 350

Leu Leu Gln Leu Gln Asn Phe Asn Thr Leu Met Ala Val Val Gly Gly
        355                 360                 365

Leu Ser His Ser Ser Ile Ser Arg Leu Lys Glu Thr His Ser His Val
    370                 375                 380

Ser Pro Glu Thr Ile Lys Leu Trp Glu Gly Leu Thr Glu Leu Val Thr
385                 390                 395                 400

Ala Thr Gly Asn Tyr Gly Asn Tyr Arg Arg Arg Leu Ala Ala Cys Val
                405                 410                 415

Gly Phe Arg Phe Pro Ile Leu Gly Val His Leu Lys Asp Leu Val Ala
            420                 425                 430

Leu Gln Leu Ala Leu Pro Asp Trp Leu Asp Pro Ala Arg Thr Arg Leu
        435                 440                 445

Asn Gly Ala Lys Met Lys Gln Leu Phe Ser Ile Leu Glu Glu Leu Ala
    450                 455                 460

Met Val Thr Ser Leu Arg Pro Pro Val Gln Ala Asn Pro Asp Leu Leu
465                 470                 475                 480

Ser Leu Leu Thr Val Ser Leu Asp Gln Tyr Gln Thr Glu Asp Glu Leu
                485                 490                 495

Tyr Gln Leu Ser Leu Gln Arg Glu Pro Arg Ser Lys Ser Ser Pro Thr
            500                 505                 510

Ser Pro Thr Ser Cys Thr Pro Pro Pro Arg Pro Pro Val Leu Glu Glu
        515                 520                 525

Trp Thr Ser Ala Ala Lys Pro Lys Leu Asp Gln Ala Leu Val Val Glu
    530                 535                 540

His Ile Glu Lys Met Val Glu Ser Val Phe Arg Asn Phe Asp Val Asp
545                 550                 555                 560

Gly Asp Gly His Ile Ser Gln Glu Glu Phe Gln Ile Ile Arg Gly Asn
                565                 570                 575

Phe Pro Tyr Leu Ser Ala Phe Gly Asp Leu Asp Gln Asn Gln Asp Gly
            580                 585                 590

Cys Ile Ser Arg Glu Glu Met Val Ser Tyr Phe Leu Arg Ser Ser Ser
        595                 600                 605

Val Leu Gly Gly Arg Met Gly Phe Val His Asn Phe Gln Glu Ser Asn
    610                 615                 620

Ser Leu Arg Pro Val Ala Cys Arg His Cys Lys Ala Leu Ile Leu Gly

```
625                 630                 635                 640

Ile Tyr Lys Gln Gly Leu Lys Cys Arg Ala Cys Gly Val Asn Cys His
                645                 650                 655

Lys Gln Cys Lys Asp Arg Leu Ser Val Glu Cys Arg Arg Arg Ala Gln
            660                 665                 670

Ser Val Ser Leu Glu Gly Ser Ala Pro Ser Pro Ser Pro Met His Ser
        675                 680                 685

His His His Arg Ala Phe Ser Phe Ser Leu Pro Arg Pro Gly Arg Arg
    690                 695                 700

Gly Ser Arg Pro Pro Glu Ile Arg Glu Glu Glu Val Gln Thr Val Glu
705                 710                 715                 720

Asp Gly Val Phe Asp Ile His Leu
                725
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2309 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 254..2083

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
CGATTTCATT CCTCGCTCCC CACAGGTCCC TCTCCCCAAA ATATTCCCAT CTTGTCCTAG      60

CCCATCCCCC AGACTATCTC AAGGACCAGC TGTCCCCACG CCCCCGACCT CCACTAGGCC     120

TGTGCCACCC GCTGCCTGCA GGAAGACGCC CGGTCCCGGG CCGGGTTAGC CCCATGGGAA     180

CGGGGTTCGG TCCGAGCCCG GTGGGAGGCT CCCGGAGCGC AGCCTGGGCC CAGCCCACCC     240

CGCGCCGGCG GCC ATG GCA GGC ACC CTG GAC CTG GAC AAG GGC TGC ACG        289
               Met Ala Gly Thr Leu Asp Leu Asp Lys Gly Cys Thr
                 1               5                  10

GTG GAG GAG CTG CTC CGC GGG TGC ATC GAA GCC TTC GAT GAC TCC GGG       337
Val Glu Glu Leu Leu Arg Gly Cys Ile Glu Ala Phe Asp Asp Ser Gly
        15                  20                  25

AAG GTG CGG GAC CCG CAG CTG GTG CGC ATG TTC CTC ATG ATG CAC CCC       385
Lys Val Arg Asp Pro Gln Leu Val Arg Met Phe Leu Met Met His Pro
    30                  35                  40

TGG TAC ATC CCC TCC TCT CAG CTG GCG GCC AAG CTG CTC CAC ATC TAC       433
Trp Tyr Ile Pro Ser Ser Gln Leu Ala Ala Lys Leu Leu His Ile Tyr
45                  50                  55                  60

CAA CAA TCC CGG AAG GAC AAC TCC AAT TCC CTG CAG GTG AAA ACG TGC       481
Gln Gln Ser Arg Lys Asp Asn Ser Asn Ser Leu Gln Val Lys Thr Cys
                65                  70                  75

CAC CTG GTC AGG TAC TGG ATC TCC GCC TTC CCA GCG GAG TTT GAC TTG       529
```

```
His Leu Val Arg Tyr Trp Ile Ser Ala Phe Pro Ala Glu Phe Asp Leu
            80                  85                  90

AAC CCG GAG TTG GCT GAG CAG ATC AAG GAG CTG AAG GCT CTG CTA GAC     577
Asn Pro Glu Leu Ala Glu Gln Ile Lys Glu Leu Lys Ala Leu Leu Asp
        95                  100                 105

CAA GAA GGG AAC CGA CGG CAC AGC AGC CTA ATC GAC ATA GAC AGC GTC     625
Gln Glu Gly Asn Arg Arg His Ser Ser Leu Ile Asp Ile Asp Ser Val
    110                 115                 120

CCT ACC TAC AAG TGG AAG CGG CAG GTG ACT CAG CGG AAC CCT GTG GGA     673
Pro Thr Tyr Lys Trp Lys Arg Gln Val Thr Gln Arg Asn Pro Val Gly
125                 130                 135                 140

CAG AAA AAG CGC AAG ATG TCC CTG TTG TTT GAC CAC CTG GAG CCC ATG     721
Gln Lys Lys Arg Lys Met Ser Leu Leu Phe Asp His Leu Glu Pro Met
                145                 150                 155

GAG CTG GCG GAG CAT CTC ACC TAC TTG GAG TAT CGC TCC TTC TGC AAG     769
Glu Leu Ala Glu His Leu Thr Tyr Leu Glu Tyr Arg Ser Phe Cys Lys
            160                 165                 170

ATC CTG TTT CAG GAC TAT CAC AGT TTC GTG ACT CAT GGC TGC ACT GTG     817
Ile Leu Phe Gln Asp Tyr His Ser Phe Val Thr His Gly Cys Thr Val
        175                 180                 185

GAC AAC CCC GTC CTG GAG CGG TTC ATC TCC CTC TTC AAC AGC GTC TCA     865
Asp Asn Pro Val Leu Glu Arg Phe Ile Ser Leu Phe Asn Ser Val Ser
    190                 195                 200

CAG TGG GTG CAG CTC ATG ATC CTC AGC AAA CCC ACA GCC CCG CAG CGG     913
Gln Trp Val Gln Leu Met Ile Leu Ser Lys Pro Thr Ala Pro Gln Arg
205                 210                 215                 220

GCC CTG GTC ATC ACA CAC TTT GTC CAC GTG GCG GAG AAG CTG CTA CAG     961
Ala Leu Val Ile Thr His Phe Val His Val Ala Glu Lys Leu Leu Gln
                225                 230                 235

CTG CAG AAC TTC AAC ACG CTG ATG GCA GTG GTC GGG GGC CTG AGC CAC     1009
Leu Gln Asn Phe Asn Thr Leu Met Ala Val Val Gly Gly Leu Ser His
            240                 245                 250

AGC TCC ATC TCC CGC CTC AAG GAG ACC CAC AGC CAC GTT AGC CCT GAG     1057
Ser Ser Ile Ser Arg Leu Lys Glu Thr His Ser His Val Ser Pro Glu
        255                 260                 265

ACC ATC AAG CTC TGG GAG GGT CTC ACG GAA CTA GTG ACG GCG ACA GGC     1105
Thr Ile Lys Leu Trp Glu Gly Leu Thr Glu Leu Val Thr Ala Thr Gly
    270                 275                 280

AAC TAT GGC AAC TAC CGG CGT CGG CTG GCA GCC TGT GTG GGC TTC CGC     1153
Asn Tyr Gly Asn Tyr Arg Arg Arg Leu Ala Ala Cys Val Gly Phe Arg
285                 290                 295                 300

TTC CCG ATC CTG GGT GTG CAC CTC AAG GAC CTG GTG GCC CTG CAG CTG     1201
Phe Pro Ile Leu Gly Val His Leu Lys Asp Leu Val Ala Leu Gln Leu
                305                 310                 315

GCA CTG CCT GAC TGG CTG GAC CCA GCC CGG ACC CGG CTC AAC GGG GCC     1249
Ala Leu Pro Asp Trp Leu Asp Pro Ala Arg Thr Arg Leu Asn Gly Ala
            320                 325                 330

AAG ATG AAG CAG CTC TTT AGC ATC CTG GAG GAG CTG GCC ATG GTG ACC     1297
Lys Met Lys Gln Leu Phe Ser Ile Leu Glu Glu Leu Ala Met Val Thr
        335                 340                 345
```

```
AGC CTG CGG CCA CCA GTA CAG GCC AAC CCC GAC CTG CTG AGC CTG CTC      1345
Ser Leu Arg Pro Pro Val Gln Ala Asn Pro Asp Leu Leu Ser Leu Leu
    350                 355                 360

ACG GTG TCT CTG GAT CAG TAT CAG ACG GAG GAT GAG CTG TAC CAG CTG      1393
Thr Val Ser Leu Asp Gln Tyr Gln Thr Glu Asp Glu Leu Tyr Gln Leu
365                 370                 375                 380

TCC CTG CAG CGG GAG CCG CGC TCC AAG TCC TCG CCA ACC AGC CCC ACG      1441
Ser Leu Gln Arg Glu Pro Arg Ser Lys Ser Ser Pro Thr Ser Pro Thr
                385                 390                 395

AGT TGC ACC CCA CCA CCC CGG CCC CCG GTA CTG GAG GAG TGG ACC TCG      1489
Ser Cys Thr Pro Pro Pro Arg Pro Pro Val Leu Glu Glu Trp Thr Ser
            400                 405                 410

GCT GCC AAA CCC AAG CTG GAT CAG GCC CTC GTG GTG GAG CAC ATC GAG      1537
Ala Ala Lys Pro Lys Leu Asp Gln Ala Leu Val Val Glu His Ile Glu
        415                 420                 425

AAG ATG GTG GAG TCT GTG TTC CGG AAC TTT GAC GTC GAT GGG GAT GGC      1585
Lys Met Val Glu Ser Val Phe Arg Asn Phe Asp Val Asp Gly Asp Gly
    430                 435                 440

CAC ATC TCA CAG GAA GAA TTC CAG ATC ATC CGT GGG AAC TTC CCT TAC      1633
His Ile Ser Gln Glu Glu Phe Gln Ile Ile Arg Gly Asn Phe Pro Tyr
445                 450                 455                 460

CTC AGC GCC TTT GGG GAC CTC GAC CAG AAC CAG GAT GGC TGC ATC AGC      1681
Leu Ser Ala Phe Gly Asp Leu Asp Gln Asn Gln Asp Gly Cys Ile Ser
                465                 470                 475

AGG GAG GAG ATG GTT TCC TAT TTC CTG CGC TCC AGC TCT GTG TTG GGG      1729
Arg Glu Glu Met Val Ser Tyr Phe Leu Arg Ser Ser Ser Val Leu Gly
            480                 485                 490

GGG CGC ATG GGC TTC GTA CAC AAC TTC CAG GAG AGC AAC TCC TTG CGC      1777
Gly Arg Met Gly Phe Val His Asn Phe Gln Glu Ser Asn Ser Leu Arg
        495                 500                 505

CCC GTC GCC TGC CGC CAC TGC AAA GCC CTG ATC CTG GGC ATC TAC AAG      1825
Pro Val Ala Cys Arg His Cys Lys Ala Leu Ile Leu Gly Ile Tyr Lys
    510                 515                 520

CAG GGC CTC AAA TGC CGA GCC TGT GGA GTG AAC TGC CAC AAG CAG TGC      1873
Gln Gly Leu Lys Cys Arg Ala Cys Gly Val Asn Cys His Lys Gln Cys
525                 530                 535                 540

AAG GAT CGC CTG TCA GTT GAG TGT CGG CGC AGG GCC CAG AGT GTG AGC      1921
Lys Asp Arg Leu Ser Val Glu Cys Arg Arg Arg Ala Gln Ser Val Ser
                545                 550                 555

CTG GAG GGG TCT GCA CCC TCA CCC TCA CCC ATG CAC AGC CAC CAT CAC      1969
Leu Glu Gly Ser Ala Pro Ser Pro Ser Pro Met His Ser His His His
            560                 565                 570

CGC GCC TTC AGC TTC TCT CTG CCC CGC CCT GGC AGG CGA GGC TCC AGG      2017
Arg Ala Phe Ser Phe Ser Leu Pro Arg Pro Gly Arg Arg Gly Ser Arg
        575                 580                 585

CCT CCA GAG ATC CGT GAG GAG GAG GTA CAG ACG GTG GAG GAT GGG GTG      2065
Pro Pro Glu Ile Arg Glu Glu Glu Val Gln Thr Val Glu Asp Gly Val
    590                 595                 600

TTT GAC ATC CAC TTG TAATAGATGC TGTGGTTGGA TCAAGGACTC ATTCCTGCCT      2120
Phe Asp Ile His Leu
605                 610
```

```
TGGAGAAAAT ACTTCAACCA GAGCAGGGAG CCTGGGGGTG TCGGGGCAGG AGGCTGGGGA    2180

TGGGGGTGGG ATATGAGGGT GGCATGCAGC TGAGGGCAGG GCCAGGGCTG GTGTCCCTAA    2240

GGTTGTACAG ACTCTTGTGA ATATTTGTAT TTTCCAGATG GAATAAAAAG GCCCGTGTAA    2300

TTAACCTTC                                                            2309
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 609 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Ala Gly Thr Leu Asp Leu Asp Lys Gly Cys Thr Val Glu Glu Leu
  1               5                  10                  15

Leu Arg Gly Cys Ile Glu Ala Phe Asp Asp Ser Gly Lys Val Arg Asp
             20                  25                  30

Pro Gln Leu Val Arg Met Phe Leu Met Met His Pro Trp Tyr Ile Pro
         35                  40                  45

Ser Ser Gln Leu Ala Ala Lys Leu Leu His Ile Tyr Gln Gln Ser Arg
     50                  55                  60

Lys Asp Asn Ser Asn Ser Leu Gln Val Lys Thr Cys His Leu Val Arg
 65                  70                  75                  80

Tyr Trp Ile Ser Ala Phe Pro Ala Glu Phe Asp Leu Asn Pro Glu Leu
                 85                  90                  95

Ala Glu Gln Ile Lys Glu Leu Lys Ala Leu Leu Asp Gln Glu Gly Asn
            100                 105                 110

Arg Arg His Ser Ser Leu Ile Asp Ile Asp Ser Val Pro Thr Tyr Lys
        115                 120                 125

Trp Lys Arg Gln Val Thr Gln Arg Asn Pro Val Gly Gln Lys Lys Arg
    130                 135                 140

Lys Met Ser Leu Leu Phe Asp His Leu Glu Pro Met Glu Leu Ala Glu
145                 150                 155                 160

His Leu Thr Tyr Leu Glu Tyr Arg Ser Phe Cys Lys Ile Leu Phe Gln
                165                 170                 175

Asp Tyr His Ser Phe Val Thr His Gly Cys Thr Val Asp Asn Pro Val
            180                 185                 190

Leu Glu Arg Phe Ile Ser Leu Phe Asn Ser Val Ser Gln Trp Val Gln
        195                 200                 205

Leu Met Ile Leu Ser Lys Pro Thr Ala Pro Gln Arg Ala Leu Val Ile
    210                 215                 220

Thr His Phe Val His Val Ala Glu Lys Leu Leu Gln Leu Gln Asn Phe
225                 230                 235                 240

Asn Thr Leu Met Ala Val Val Gly Gly Leu Ser His Ser Ser Ile Ser
                245                 250                 255
```

```
Arg Leu Lys Glu Thr His Ser His Val Ser Pro Glu Thr Ile Lys Leu
            260                 265                 270

Trp Glu Gly Leu Thr Glu Leu Val Thr Ala Thr Gly Asn Tyr Gly Asn
        275                 280                 285

Tyr Arg Arg Arg Leu Ala Ala Cys Val Gly Phe Arg Phe Pro Ile Leu
    290                 295                 300

Gly Val His Leu Lys Asp Leu Val Ala Leu Gln Leu Ala Leu Pro Asp
305                 310                 315                 320

Trp Leu Asp Pro Ala Arg Thr Arg Leu Asn Gly Ala Lys Met Lys Gln
                325                 330                 335

Leu Phe Ser Ile Leu Glu Glu Leu Ala Met Val Thr Ser Leu Arg Pro
            340                 345                 350

Pro Val Gln Ala Asn Pro Asp Leu Leu Ser Leu Leu Thr Val Ser Leu
        355                 360                 365

Asp Gln Tyr Gln Thr Glu Asp Glu Leu Tyr Gln Leu Ser Leu Gln Arg
    370                 375                 380

Glu Pro Arg Ser Lys Ser Ser Pro Thr Ser Pro Thr Ser Cys Thr Pro
385                 390                 395                 400

Pro Pro Arg Pro Pro Val Leu Glu Glu Trp Thr Ser Ala Ala Lys Pro
                405                 410                 415

Lys Leu Asp Gln Ala Leu Val Val Glu His Ile Glu Lys Met Val Glu
            420                 425                 430

Ser Val Phe Arg Asn Phe Asp Val Asp Gly Asp Gly His Ile Ser Gln
        435                 440                 445

Glu Glu Phe Gln Ile Ile Arg Gly Asn Phe Pro Tyr Leu Ser Ala Phe
    450                 455                 460

Gly Asp Leu Asp Gln Asn Gln Asp Gly Cys Ile Ser Arg Glu Glu Met
465                 470                 475                 480

Val Ser Tyr Phe Leu Arg Ser Ser Ser Val Leu Gly Gly Arg Met Gly
                485                 490                 495

Phe Val His Asn Phe Gln Glu Ser Asn Ser Leu Arg Pro Val Ala Cys
            500                 505                 510

Arg His Cys Lys Ala Leu Ile Leu Gly Ile Tyr Lys Gln Gly Leu Lys
        515                 520                 525

Cys Arg Ala Cys Gly Val Asn Cys His Lys Gln Cys Lys Asp Arg Leu
    530                 535                 540

Ser Val Glu Cys Arg Arg Arg Ala Gln Ser Val Ser Leu Glu Gly Ser
545                 550                 555                 560

Ala Pro Ser Pro Ser Pro Met His Ser His His His Arg Ala Phe Ser
                565                 570                 575

Phe Ser Leu Pro Arg Pro Gly Arg Arg Gly Ser Arg Pro Pro Glu Ile
            580                 585                 590

Arg Glu Glu Glu Val Gln Thr Val Glu Asp Gly Val Phe Asp Ile His
        595                 600                 605

Leu
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 11..733

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
GCCCGCCGCC ATG CCG CCC TTA CTG CCC CTG CGC CTG TGC CGG CTG TGG          49
           Met Pro Pro Leu Leu Pro Leu Arg Leu Cys Arg Leu Trp
             1               5                  10

CCC CGC AAC CCT CCC TCC CGG CTC CTC GGA GCG GCC GCC GGG CAG CGG         97
Pro Arg Asn Pro Pro Ser Arg Leu Leu Gly Ala Ala Ala Gly Gln Arg
     15                  20                  25

TCC AGA CCC AGT ACT TAT TAT GAA CTG TTG GGG GTG CAT CCT GGT GCC        145
Ser Arg Pro Ser Thr Tyr Tyr Glu Leu Leu Gly Val His Pro Gly Ala
 30                  35                  40                  45

AGC ACT GAG GAA GTT AAA CGA GCT TTC TTC TCC AAG TCC AAA GAG CTG        193
Ser Thr Glu Glu Val Lys Arg Ala Phe Phe Ser Lys Ser Lys Glu Leu
                 50                  55                  60

CAC CCA GAC CGG GAC CCT GGG AAC CCA AGC CTG CAC AGC CGC TTT GTG        241
His Pro Asp Arg Asp Pro Gly Asn Pro Ser Leu His Ser Arg Phe Val
             65                  70                  75

GAG CTG AGC GAG GCA TAC CGT GTG CTC AGC CGT GAG CAG AGC CGC CGC        289
Glu Leu Ser Glu Ala Tyr Arg Val Leu Ser Arg Glu Gln Ser Arg Arg
         80                  85                  90

AGC TAT GAT GAC CAG CTC CGC TCA GGT AGT CCC CCA AAG TCT CCA CGA        337
Ser Tyr Asp Asp Gln Leu Arg Ser Gly Ser Pro Pro Lys Ser Pro Arg
     95                 100                 105

ACC ACA GTC CAT GAC AAG TCT GCC CAC CAA ACA CAC AGC TCC TGG ACA        385
Thr Thr Val His Asp Lys Ser Ala His Gln Thr His Ser Ser Trp Thr
110                 115                 120                 125

CCC CCC AAC GCA CAG TAC TGG TCC CAG TTT CAC AGC GTG AGG CCA CAG        433
Pro Pro Asn Ala Gln Tyr Trp Ser Gln Phe His Ser Val Arg Pro Gln
                130                 135                 140

GGG CCC CAG TTG AGG CAG CAG CAA CAC AAA CAA AAC AAA CAA GTG CTG        481
Gly Pro Gln Leu Arg Gln Gln Gln His Lys Gln Asn Lys Gln Val Leu
            145                 150                 155

GGG TAC TGC CTC CTC CTC ATG CTG GCG GGC ATG GGC CTG CAC TAC ATT        529
Gly Tyr Cys Leu Leu Leu Met Leu Ala Gly Met Gly Leu His Tyr Ile
        160                 165                 170

GCC TTC AGG AAG GTG AAG CAG ATG CAC CTT AAC TTC ATG GAT GAA AAG        577
Ala Phe Arg Lys Val Lys Gln Met His Leu Asn Phe Met Asp Glu Lys
    175                 180                 185
```

```
GAT CGG ATC ATC ACA GCC TTC TAC AAC GAA GCC CGG GCA CGG GCC AGG         625
Asp Arg Ile Ile Thr Ala Phe Tyr Asn Glu Ala Arg Ala Arg Ala Arg
190                 195                 200                 205

GCC AAC AGA GGC ATC CTT CAG CAG GAG CGA CAA CGG CTA GGG CAG CGG         673
Ala Asn Arg Gly Ile Leu Gln Gln Glu Arg Gln Arg Leu Gly Gln Arg
                210                 215                 220

CAG CCG CCA CCA TCC GAG CCA ACC CAA GGC CCC GAG ATC GTG CCC CGG         721
Gln Pro Pro Pro Ser Glu Pro Thr Gln Gly Pro Glu Ile Val Pro Arg
            225                 230                 235

GGC GCC GGC CCC TGA GGGGCTC ACCTGGATGG GGCCTGCAGT GCGTTCCCGC            773
Gly Ala Gly Pro  *
        240

TTTGCTTCCT TCCCTGGACG GCCCGCTCCC CGAAACGCGC GCAATAAAGT GATTCGCAG        832
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 241 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Pro Pro Leu Leu Pro Leu Arg Leu Cys Arg Leu Trp Pro Arg Asn
 1               5                  10                  15

Pro Pro Ser Arg Leu Leu Gly Ala Ala Ala Gly Gln Arg Ser Arg Pro
            20                  25                  30

Ser Thr Tyr Tyr Glu Leu Leu Gly Val His Pro Gly Ala Ser Thr Glu
        35                  40                  45

Glu Val Lys Arg Ala Phe Phe Ser Lys Ser Lys Glu Leu His Pro Asp
    50                  55                  60

Arg Asp Pro Gly Asn Pro Ser Leu His Ser Arg Phe Val Glu Leu Ser
65                  70                  75                  80

Glu Ala Tyr Arg Val Leu Ser Arg Glu Gln Ser Arg Arg Ser Tyr Asp
                85                  90                  95

Asp Gln Leu Arg Ser Gly Ser Pro Pro Lys Ser Pro Arg Thr Thr Val
            100                 105                 110

His Asp Lys Ser Ala His Gln Thr His Ser Ser Trp Thr Pro Pro Asn
        115                 120                 125

Ala Gln Tyr Trp Ser Gln Phe His Ser Val Arg Pro Gln Gly Pro Gln
    130                 135                 140

Leu Arg Gln Gln Gln His Lys Gln Asn Lys Gln Val Leu Gly Tyr Cys
145                 150                 155                 160

Leu Leu Leu Met Leu Ala Gly Met Gly Leu His Tyr Ile Ala Phe Arg
                165                 170                 175

Lys Val Lys Gln Met His Leu Asn Phe Met Asp Glu Lys Asp Arg Ile
            180                 185                 190

Ile Thr Ala Phe Tyr Asn Glu Ala Arg Ala Arg Ala Arg Ala Asn Arg
```

```
        195                 200                 205

Gly Ile Leu Gln Gln Glu Arg Gln Arg Leu Gly Gln Arg Gln Pro Pro
    210                     215                 220

Pro Ser Glu Pro Thr Gln Gly Pro Glu Ile Val Pro Arg Gly Ala Gly
225                 230                     235                 240

Pro
```

SEQ ID Nos: 10-18 25-36

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 300 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 170..300

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CGATTTCATT CCTCGCTCCC CACAGGTCCC TCTCCCCAAA ATATTCCCAT CTTGTCCTAG      60

CCCATCCCCC AGACTATCTC AAGGACCAGC TGTCCCCACG CCCCCGACCT CCACTAGGCC     120

TGTGCCACCC GCTGCCTGCA GGAAGACGCC CGGTCCCGGG CCGGGTTAG CCC CAT         175
                                                      Pro His
                                                        1

GGG AAC GGG GTT CGG TCC GAG CCC GGT GGG AGG CTC CCG GAG CGC AGC       223
Gly Asn Gly Val Arg Ser Glu Pro Gly Gly Arg Leu Pro Glu Arg Ser
         5                  10                  15

CTG GGC CCA GCC CAC CCC GCG CCG GCG GCC ATG GCA GGC ACC CTG GAC       271
Leu Gly Pro Ala His Pro Ala Pro Ala Ala Met Ala Gly Thr Leu Asp
     20                  25                  30

CTG GAC AAG GGC TGC ACG GTG GAG GAG CT                                300
Leu Asp Lys Gly Cys Thr Val Glu Glu Leu
 35                  40
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro His Gly Asn Gly Val Arg Ser Glu Pro Gly Gly Arg Leu Pro Glu
1 5 10 15

Arg Ser Leu Gly Pro Ala His Pro Ala Pro Ala Ala Met Ala Gly Thr
20 25 30

Leu Asp Leu Asp Lys Gly Cys Thr Val Glu Glu Leu
35 40

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGATCCCCC TGGTC 15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Val Asp Glu Glu Asp Glu Val Glu Asp Ile Glu Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Asp Val Asp Gly Asp Gly His Ile Ser Gln Glu Glu Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Asp His Asp Arg Asp Gly Phe Ile Ser Gln Glu Glu Phe
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Asp Gln Asn Gln Asp Gly Cys Ile Ser Arg Glu Glu Met
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Asp Val Asp Met Asp Gly Gln Ile Ser Lys Asp Glu Leu
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
His Phe Val His Val Ala Glu Lys Leu Leu Gln Leu Gln Asn Phe Asn
1               5                   10                  15

Thr Leu Met Ala Val Val Gly Gly Leu Ser His Ser Ser Ile Ser Arg
            20                  25                  30
```

Leu Lys Glu Thr His
35

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Lys Phe Val His Val Ala Lys His Leu Arg Lys Ile Asn Asn Phe Asn
1 5 10 15

Thr Leu Met Ser Val Val Gly Gly Ile Thr His Ser Ser Val Ala Arg
20 25 30

Leu Ala Lys Thr Tyr
35

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His Asn Phe Gln Glu Ser Asn Ser Leu Arg Pro Val Ala Cys Arg His
1 5 10 15

Cys Lys Ala Leu Ile Leu Gly Ile Tyr Lys Gln Gly Leu Lys Cys Arg
20 25 30

Ala Cys Gly Val Asn Cys His Lys Gln Cys Lys Asp Arg Leu Ser Val
35 40 45

Glu Cys
50

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Asn Phe His Glu Thr Thr Phe Leu Thr Pro Thr Thr Cys Asn His

```
1               5                   10                  15
Cys Asn Lys Leu Leu Trp Gly Ile Leu Arg Gln Gly Phe Lys Cys Lys
            20                  25                  30
Asp Cys Gly Leu Ala Val His Ser Cys Cys Lys Ser Asn Ala Val Ala
        35                  40                  45
Glu Cys
    50
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGATCCCCC TGGTC 15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GAATTCGGCA CGAGCCGACG G 21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ATGGAGCAGA AGCTGATCTC CGAGGAGGAC CTGCCCGGGG CAGCTGGATC CGCAGCCCAC 60

CCCGCGCCGG CGGCCATG 78

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Pro Gly Ala Ala Gly
1               5                   10                  15

Ser Ala Ala His Pro Ala Pro Ala Ala Met
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
GGATCCGCAG CCCACCCCGC GCCGGCGGCC ATG                                    33
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Gly Ser Ala Ala His Pro Ala Pro Ala Ala Met
                5                   10
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGACAAAGTG TGTGATGAAC C 21

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTCATCCTCC GTCTGATACT G 21

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTAGATGTGG ATCAGCTTGG 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGGTGGAGAA TGGTCAAGG 19

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTCATAGTCT GTCTCCTACT 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ACATAGACAG CGTGCCTACC 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TACAACCTTA GGGACACCAG 20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TGCTGAGCCT GCTCACGGTG 20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CAAGTGAACA GCACGTCC 18

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GACTATCTCA AGGACCAGCT G 21

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGTTCGGTCC GAGCCCGG 18

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGAGCGATAC TCCAAGTAGG T 21

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

AGCGGGCCAG GCCCCTTC 18

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CATCCTGGTC CAATGCGCTC 20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GCACTGAGGA AGTTAAACGA GC 22

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GCTCGTTTAA CTTCCTCAGT GC 22

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GCTCAGCTCC ACAAAGCGGC T 21

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

ACCAGCTCCG CTCAGGTAG 19

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TCCAGGAGCT GTGTGTTTGG 20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCAGTTTCAC AGCGTGAGG 19

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAGCATGAGG AGGAGGCAG 19

CLAIMS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a regulator of gene expression or a derivative of said gene regulator.

2. An isolated nucleic acid molecule according to claim 1 wherein the regulator comprises a zinc finger domain of an $(HC_3)_2$ type.

3. An isolated nucleic acid molecule according to claim 2 wherein the sequence of nucleotides or complementary sequence of nucleotides is selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:2;
(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:3;
(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
(iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

4. An isolated nucleic acid molecule according to claim 1 wherein said gene regulator is a guanine nucleotide exchange factor (GEF) or a derivative thereof.

5. An isolated nucleic acid molecule according to claim 4 wherein the sequence of nucleotides is selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:4 or 6;
(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:5 or 7;
(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
(iv) a nucleotide sequence capable of hybridising under low stringency conditions to the

nucleotide sequence set forth in (i), (ii) or (iii).

6. An isolated nucleic acid molecule according to claim 1, wherein said gene regulator is a heat shock protein or is a heat shock binding protein or a derivative thereof.

7. An isolated nucleic acid molecule according to claim 6, wherein the sequence of nucleotides is selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:8;

(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:9;

(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and

(iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

8. A genetic construct comprising a vector portion and a gene portion comprising a regulator of gene expression or a derivative thereof .

9. A genetic construct according to claim 8 wherein the gene portion comprises a zinc finger domain of $(HC_3)_2$ type.

10. A genetic construct according to claim 9 wherein the gene portion comprises a nucleotide sequence selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:2;

(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:3;

(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and

(iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

11. A genetic construct according to claim 8 wherein said gene portion is a nucleotide exchange factor (GEF) or derivative thereof.

12. A genetic construct according to claim 11 wherein the gene portion comprises a nucleotide sequence selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:4 or 6;
(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:5 or 7;
(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
(iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

13. A genetic construct according to claim 8 wherein the gene portion is a heat shock protein or a derivative thereof or a heat shock binding protein or derivative thereof.

14. A genetic construct according to claim 13 wherein the gene portion comprises a nucleotide sequence selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:8;
(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:9;
(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and
(iv) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

15. A nucleic acid molecule encoding a gene regulator having the identifying characteristics of a molecule selected from MCG4, MCG7 and MCG18 having respective amino acid sequences of SEQ ID NO:3, SEQ ID NO: 5 or 7 and SEQ ID NO:9.

16. A method of detecting a condition caused or facilitated by an aberration in *mcg4*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg4* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

17. A method of detecting a condition caused or facilitated by an aberration in *mcg4*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG4 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

18. A method for detecting MCG4 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG4 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG4 complex to form, and then detecting said complex.

19. A method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg7* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

20. A method of detecting a condition caused or facilitated by an aberration in *mcg7*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG7 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

21. A method for detecting MCG7 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG7 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG7 complex to form, and then detecting said complex.

22. A method of detecting a condition caused or facilitated by an aberration in *mcg18*, said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg18* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

23. A method of detecting a condition caused or facilitated by an aberration in *mcg18*, said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG18 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

24. A method for detecting MCG18 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG18 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG18 complex to form, and then detecting said complex.

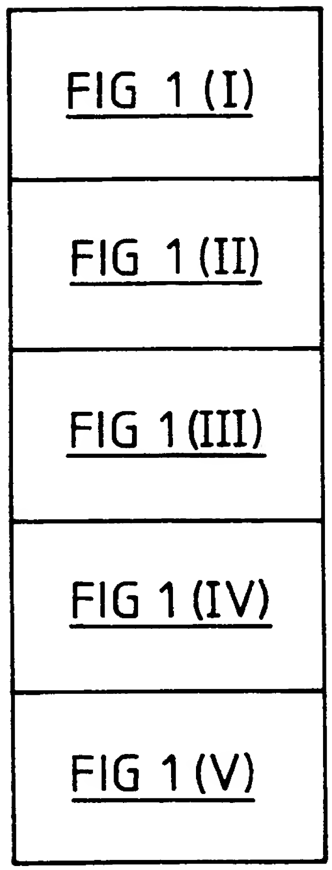


FIG 1

**FIGURE 1 (I)**

```
TCAGTAAACA CAGAGACTGG GGATCGATC ATG GGG CTT TGT AAG TGC CCC   50
                                Met Gly Leu Cys Lys Cys Pro
                                 1               5

AAG AGA AAG GTG ACC AAC CTG TTC TGC TTC GAA CAT CGG GTC AAC   95
Lys Arg Lys Val Thr Asn Leu Phe Cys Phe Glu His Arg Val Asn
         10                  15                  20

GTC TGC GAG CAC TGC CTG GTA GCC AAT CAC GCC AAG TGC ATC GTC  140
Val Cys Glu His Cys Leu Val Ala Asn His Ala Lys Cys Ile Val
         25                  30                  35

CAG TCC TAC CTG CAA TGG CTC CAA GAT AGC GAC TAC AAC CCC AAT  185
Gln Ser Tyr Leu Gln Trp Leu Gln Asp Ser Asp Tyr Asn Pro Asn
         40                  45                  50

TGC CGC CTG TGC AAC ATA CCC CTG GCC AGC CGA GAG ACG ACC CGC  230
Cys Arg Leu Cys Asn Ile Pro Leu Ala Ser Arg Glu Thr Thr Arg
         55                  60                  65

CTT GTC TGC TAT GAT CTC TTT CAC TGG GCC TGC CTC AAT GAA CGT  275
Leu Val Cys Tyr Asp Leu Phe His Trp Ala Cys Leu Asn Glu Arg
         70                  75                  80
```

**FIGURE 1 (II)**

```
GCT GCC CAG CTA CCC CGA AAC ACG GCA CCT GCC GGC TAT CAG TGC  320
Ala Ala Gln Leu Pro Arg Asn Thr Ala Pro Ala Gly Tyr Gln Cys
         85                  90                  95

CCC AGC TGC AAT GGC CCC ATC TTC CCC CCA ACC AAC CTG GCT GGC  365
Pro Ser Cys Asn Gly Pro Ile Phe Pro Pro Thr Asn Leu Ala Gly
        100                 105                 110

CCC GTG GCC TCC GCA CTG AGA GAG AAG CTG GCC ACA GTC AAC TGG  410
Pro Val Ala Ser Ala Leu Arg Glu Lys Leu Ala Thr Val Asn Trp
        115                 120                 125

GCC CGG GCA GGA CTG GGC CTC CCT CTG ATC GAT GAG GTG GTG AGC  455
Ala Arg Ala Gly Leu Gly Leu Pro Leu Ile Asp Glu Val Val Ser
        130                 135                 140

CCA GAG CCC GAG CCC CTC AAC ACG TCT GAC TTC TCT GAC TGG TCT  500
Pro Glu Pro Glu Pro Leu Asn Thr Ser Asp Phe Ser Asp Trp Ser
        145                 150                 155

AGT TTT AAT GCC AGC AGT ACC CCT GGA CCA GAG GAG GTA GAC AGC  545
Ser Phe Asn Ala Ser Ser Thr Pro Gly Pro Glu Glu Val Asp Ser
        160                 165                 170
```

**FIGURE 1 (III)**

```
GCC TCT GCT GCC CCA GCC TTC TAC AGC CGA GCC CCC CGG CCC CCA  590
Ala Ser Ala Ala Pro Ala Phe Tyr Ser Arg Ala Pro Arg Pro Pro
        175                 180                 185

GCT TCC CCA GGC CGG CCC GAG CAG CAC ACA GTG ATC CAC ATG GGC  635
Ala Ser Pro Gly Arg Pro Glu Gln His Thr Val Ile His Met Gly
        190                 195                 200

AAT CCT GAG CCC TTG ACT CAC GCC CCT AGG AAG GTG TAT GAT ACG  680
Asn Pro Glu Pro Leu Thr His Ala Pro Arg Lys Val Tyr Asp Thr
        205                 210                 215

CGG GAT GAT GAC CGG ACA CCA GGC CTC CAT GGA GAC TGT GAC GAT  725
Arg Asp Asp Asp Arg Thr Pro Gly Leu His Gly Asp Cys Asp Asp
        220                 225                 230

GAC AAG TAC CGA CGT CGG CCG GCC TTG GGT TGG CTG GCC CGG CTG  770
Asp Lys Tyr Arg Arg Arg Pro Ala Leu Gly Trp Leu Ala Arg Leu
        235                 240                 245

CTA AGG AGC CGG GCT GGG TCT CGG AAG CGG CCG CTG ACC CTG CTC  815
Leu Arg Ser Arg Ala Gly Ser Arg Lys Arg Pro Leu Thr Leu Leu
        250                 255                 260
```

**FIGURE 1 (IV)**

```
CAG CGG GCG GGG CTG CTG CTA CTC TTG GGA CTG CTG GGC TTC CTG   860
Gln Arg Ala Gly Leu Leu Leu Leu Leu Gly Leu Leu Gly Phe Leu
        265                 270                 275

GCC CTC CTT GCC CTC ATG TCT CGC CTA GGC CGG GCC GCA GCT GAC   905
Ala Leu Leu Ala Leu Met Ser Arg Leu Gly Arg Ala Ala Ala Asp
        280                 285                 290

AGC GAT CCC AAC CTG GAC CCA CTC ATG AAC CCT CAC ATC CGC GTG   950
Ser Asp Pro Asn Leu Asp Pro Leu Met Asn Pro His Ile Arg Val
        295                 300                 305

GGC CCC TCC TGA GCCCCCTTGC TTGTGGCTAG GCCAGCCTAG GATGTGGGTT 1002
Gly Pro Ser  *
        310

CTGTGGAGGA GAGGCGGGGT AATGGGGAGG CTGAGGGCAC CTCTTCACTG        1052

CCCCTCTCCC TCAAGCCTAA GACACTAAGA CCCCAGACCC AAAGCCAAGT        1102

CCACCAGAGT GGCTCGCAGG CCAGGCCTGG AGTCCCCGTG GGTCAAGCAT        1152
```

**FIGURE 1 (V)**

TTGTCTTGAC TTGCTTTCTC CCGGGTCTCC AGCCTCCGAC CCCTCGCCCC 1202

ATGAAGGAGC TGGCAGGTGG AAATAAACAA CAACTTTATT 1242

**FIGURE 2**

```
gb|AA155210|AA155210 mr98e01.rl Stratagene mouse embryonic carcinoma
            (#937317)Mus musculus cDNA clone 605496 5'

Query:  1  MGLCKCPKRKVTNLFCFEHRVNVCEHCLVANHAKCIVQSYLQWLQDSDYNPNCRLCNIPL  60
           MGLCKCPKRKVTNLFCFEHRVNVCEHCLVANHAKCIVQSYLQWLQDSDYNPNCRLCN PL
Sbjct: 98  MGLCKCPKRKVTNLFCFEHRVNVCEHCLVANHAKCIVQSYLQWLQDSDYNPNCRLCNTPL 277
```

**FIGURE 3**

```
dbj|D75913|CELK111G3F C. elegans cDNA clone yk111g3:5' end, single read.

Query:    7 PKRKVTNLFCFEHRVNVCEHCLVANHAKCIVQSYLQWLQDSDYNPNCRLCNIPLASRETT  66
            PKRKVTNLF +EHRVNVCE  LV NH  C+VQSYL WL D DY+PNC LC   L   +T
Sbjct:    1 PKRKVTNLFXYEHRVNVCELXLVDNHPNCVVQSYLTWLTDQDYDPNCSLCKTTLXEGDTI 180

Query:   67 RLVCYDLFHWACLNERAAQLPRNTAPAGYQCP 98     98 PSCNGPIFPPNQ  109
            RL C  L HW C +E     P  TAP GY+CP           P C+   +FPP+Q
Sbjct: 181 RLNCLHLLHWKCFDEWXGNFPDTTAPXGYRCP 276   275 PCCSQEVFPPDQ  310
```

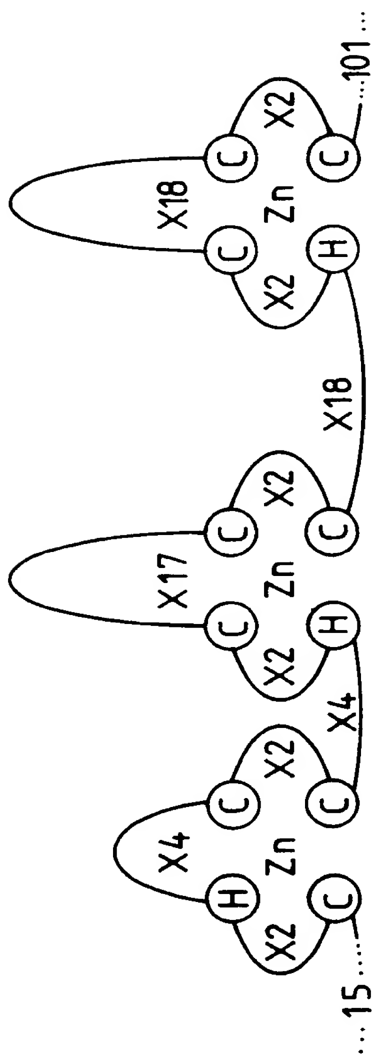


FIG 4

**FIGURE 5**

```
sp|P46580| YLBS_CAEEL HYPOTHETICAL 146.8 KD PROTEIN C34E10.5 IN
             CHROMOSOME III gi|500728 (U10402) C34E10.5 gene product
             [Caenorhabditis elegans]

Query:   56  CNIPLASRETTRLVCYDLFHWACLNERAAQLPRNTAPAGYQCPSC  100
             C+I L ++  + L C  LF W C+ E A      + + +  +CP C
Sbjct:1222   CSICLENKNPSALFCGHLFCWTCIQEHAVAATSSASTSSARCPQC 1266
```

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**FIGURE 6**

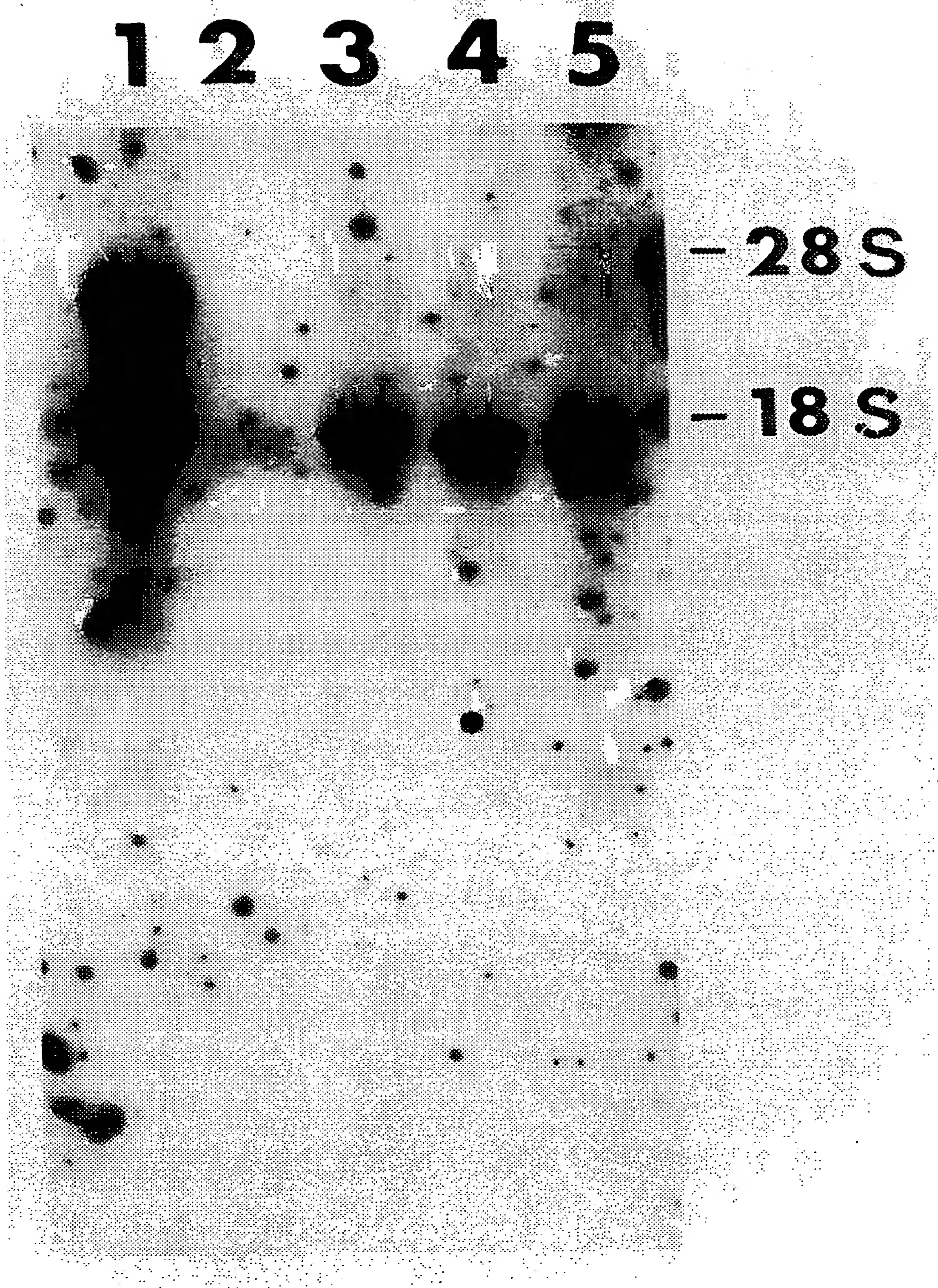
```
gi|703468| (L29051) homologous to GATA-binding transcription factor
           [Schizosaccharomyces pombe]

Query:  35  CIVQSYLQWLQDSDYNPNCRLCNI   58
            C   +  +W +D  NP C  C +
Sbjct: 175  CATTNTPKWRRDESGNPICNACGL   198

Query: 162  SSTPGPEEVDSASAAPAFYSQAPRPPASPGRPEQHTVIHMGNPEPLTHAPRKVYDTRDDD 221
            +S  PEE  S S        S  P+    SP+ +Q  +I             P +V  +  D
Sbjct: 441  ASLLNPEEPPSNSDKQPSMSNGPKSEVSPSQSQQAPLIQSSTSPVSLQFPPEVQGSNVDK 500

Query: 222  RTPGLH 227
            R  L+
Sbjct: 501  RNYALN 506
```

FIG 7



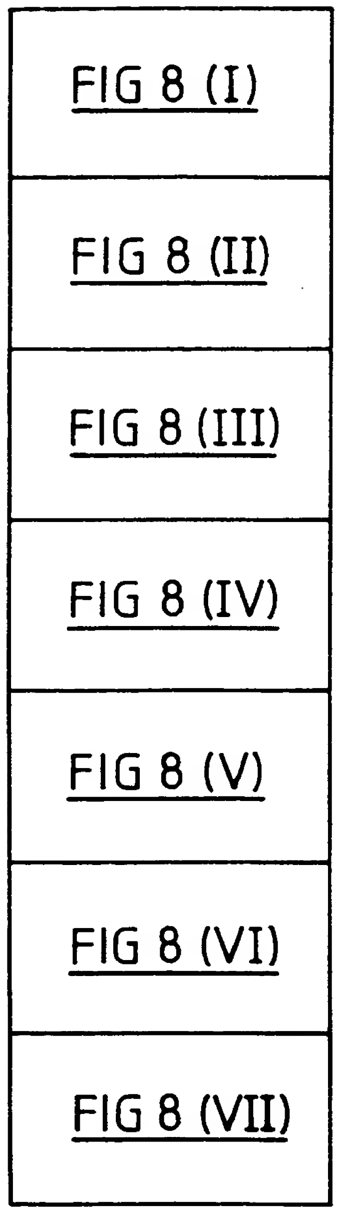



FIG 8

**FIGURE 8 (I)**

```
gb|AA074703|AA074703 zm76g07.rl Stratagene neuroepithelium (#937231)
            Homo sapiens cDNA clone 531612 5'
            Length = 417

  Plus Strand HSPs:
 Score = 818 (226.0 bits), Expect = 6.1e-103, Sum P(5)=6.1e-103
 Identities = 206/259 (79%), Positives = 206/259 (79%), Strand = Plus/Plus

Query: 446 GGCCTCCCTCTGATCGATGAGGTGGTGAGCCCAGAGCCCGAGCCCCTCAACACGTCTGAC 505
           || ||||||||||||||||||||| | |||||||||||||||||||||||  | || |||
Sbjct:  49 GGGCTCCCTCTGATCGATGAGGTGATAAGCCCAGAGCCCGAGCCCCTCAATTCCTCAGAC 108

Query: 506 TTCTCTGACTGGTCTAGTTTTAATGCCAGCAGTACCCCTGGACCAGAGGAGGTAGACAGC 565
           |||||||| ||||| || |||||||||| ||  ||| |||  | |||||||  || ||||
Sbjct: 109 TTCTCTGATTGGTCCAGCTTTAATGCCACCACCACCTCTGTGCAAGAGGAGAGAGCCAGC 168
```

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**FIGURE 8(II)**

```
Query: 566 GCCTCTGCTGCCCCAGCCTTCTACAGCCAGGCCCCCGGCCCCCAGCTTCCCCAGGCCGG 625

Sbjct: 169 ACTCCATCTGCACCTGCTTTCTATAGCCAGGCTCCCCGCCCTCCTCCCTCCCCAAGCCGT 228

Query: 626 CCCGAGCAGCACACAGTGATCCACATGGCAATCCTGAGCCCTTGACTCACGCCCCTAGG 685

Sbjct: 229 CCCGAGCAGCACACAGTCATACACATGGGAGTACTGAAGCCCTGGCACACGCCCCAAGG 288

Query: 686 AAGGTGTATGATACGCGGG 704

Sbjct: 289 AAAGTATATGACACACCGG 307
```

Score = 230 (63.6 bits), Expect = 6.1e-103, Sum P(5)=6.1e-103
Identities = 50/55 (90%), Positives = 50/55 (90%), Strand = Plus/Plus

**FIGURE 8 (III)**

```
Query: 398 GCACTGAGAGAGAAGCTGGCCACAGTCAACTGGGCCCGGCAGGACTGGGCCTCC      452
           ||||||||||| ||||| ||||||||||||| ||||||||||||||||||  ||
Sbjct:   2 GCACTGAGAGAAAAGCTAGCCACAGTCAACTTGGCCCGGCAGGACTGGGCTCCC       56
```

Score = 175(48.4 bits), Expect = 6.1e-103, Sum P(5)=6.1e-103
Identities = 39/44 (88%), Positives = 39/44 (88%), Strand = Plus/Plus

```
Query: 767 GCCTTGGGTTGGCTGGCCCGGCTGCTAAGGAGCCGGGCTGGGTC 810
           ||  |||| |||||||||| |||||| |||||||||||||||||
Sbjct: 373 GCTCTGGGCTGGCTGGCCCAGCTGCTCAGGAGCCGGGCTGGGTC 416
```

Score = 139 (38.4 bits), Expect = 6.1e-103, Sum P(5)=6.1e-103
Identities = 31/35 (88%), Positives = 31/35 (88%), Strand = Plus/Plus

**FIGURE 8 (IV)**

```
Query: 731 GGAGACTGTGACGATGACAAGTACCGACGTCGGCC 765
           ||||||||||| |||||||| ||||| || |||||
Sbjct: 336 GGAGACTGTGATGATGACAAATACCGCCGCCGGCC 370
```

Score = 133 (36.8 bits), Expect = 6.1e-103, Sum P(5)=5.1e-103
Identities = 29/32 (90%), Positives = 29/32 (90%), Strand = Plus/Plus

```
Query: 701 CGGGATGATGACCGGACACCAGGCCTCCATGG 732
           |||||||||||||||||| ||||| | |||||
Sbjct: 305 CGGGATGATGACCGGACAGCAGGCATTCATGG 336
```

**FIGURE 8 (V)**

```
gb|AA134788| AA134788 zm81g02.rl Stratagene neuroepithelium (#937231)
             Homo sapiens cDNA clone 532082 5'
             Length = 368

   Plus Strand HSPs:
 Score = 563 (155.6 bits), Expect = 3.8e-87, Sum P(3)=3.8e-87
 Identities = 147/190 (77%), Positives = 147/190 (77%), Strand = Plus/Plus

Query: 498 CGTCTGACTTCTCTGACTGGTCTAGTTTTAATGCCAGCAGTACCCCTGGACCAGAGGAGG 557
           | || |||||||||| ||||| || |||||||||| ||  ||| ||||  | |||||||
Sbjct: 103 CCTCAGACTTCTCTGATTGGTCCAGCTTTAATGCCACCACCACCTCTGTGCAAGAGGAGA 162
```

**FIGURE 8 (VI)**

```
Query: 558 TAGACAGCGCCTCTGCTGCCCCAGCCTTCTACAGCCAGGCCCCCGGCCCCCAGCTTCCC 617
            || |||| |  |  |||| || || ||||| |||||||| ||||| || ||  | ||||
Sbjct: 163 GAGCCAGCACTCCATCTGCGCCTGCTTTCTATAGCCAGGCTCCCCGCCCTCCTCCCTCCC 222

Query: 618 CAGGCCGGCCCGAGCAGCACACAGTGATCCACATGGCAATCCTGAGCCCTTGACTCACG 677
           || |||| ||||||||||||||||| || ||||||| | | ||||  || || | ||||
Sbjct: 223 CAAGCCGTCCCGAGCAGCACACAGTCATACACATGGGGAGTACTGAAGCCCTGGCACACG 282

Query: 678 CCCCTAGGAA 687
           |||| |||||
Sbjct: 283 CCCCAAGGAA 292
```

Score = 454 (125.4 bits), Expect = 3.8e-87, Sum P(3)= 3.8e-87
Identities = 94/98 (95%), Positives = 94/98 (95%), Strand = Plus/Plus

**FIGURE 8 (VII)**

```
Query: 398 GCACTGAGAGAGAAGCTGGCCACAGTCAACTGGGCCCGGGCAGGACTGGGCCTCCCTCTG 457
           ||||||||||| ||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct:   2 GCACTGAGAGACAAGCTAGCCACAGTCAACTGGGCCCGGGCAGGACTGGGCCTCCCTCTG  61

Query: 458 ATCGATGAGGTGGTGAGCCCAGAGCCCGAGCCCCTCAA 495
           |||||||||||| | |||||||||||||||||||||||
Sbjct:  62 ATCGATGAGGTGATAAGCCCAGAGCCCGAGCCCCTCAA 99
```

Score = 219 (60.5 bits), Expect = 3.8e-87, Sum P(3)= 3.8e-87
Identities = 51/60 (85%), Positives = 51/60 (85%), Strand = Plus/Plus

```
Query:702 GGATGATGACCGGACACCAGGCCTCCATGGAGACTGTGACGATGACAAGTACCGACGTC 761
          ||   ||||||||||||| ||||| | |||||||||||||| |||||||| ||||| ||| |
Sbjct:309 GGATTGATGACCGGACAGCAGGCATTCATGGAGACTGTGATGATGACAAATACCGCCGCC 368
```

**FIGURE 9**

W32939 human

TACCGCCCTTCGGAACCAGTGCAGCGGCCGATCAGTAAACACAGAGACTGGGGATCGATC<u>ATG</u>GGGCTTTGTAAG

AA242159 mouse

CTTCCGCGCTTTTCATTACCGTACGCACCGGTCA-CGATCGGCATCGCGGAGGATCGGTC<u>ATG</u>GGACTTTGCAAG

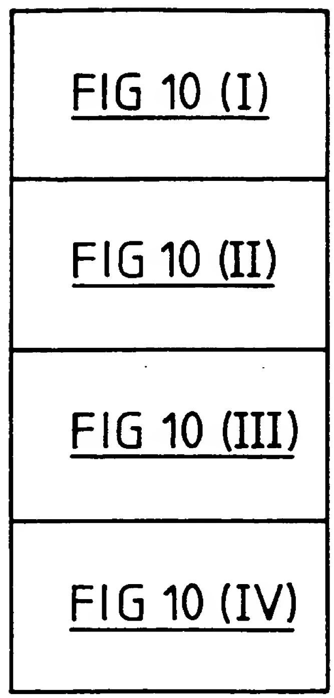


FIG 10

**FIGURE 10(I)**

```
MCG4        MGLCKCPKRK VTNLFCFEHR VNVCEHCLVA NHAKCIVQSY LQWLQDSDYN PNCRLCNIPL 60
MCG4        ASRETTRLVC YDLFHWACLN ERAAQLPRNT APAGYQCPSC NGPIFPPTNL AGPVASALRE 120
  3.
[  229 ]    ___________________________________________________________***x>
  5.
[   74 ]    ___________________________________________________________****>

                  130        140        150        160        170        180
                    *          *          *          *          *          *
MCG4        KLATVNWARA GLGLPLIDEV VSPEPEPLNT SDFSDWSSFN ASSTPGPEEV DSASAAPAFY
  1.                         20         30         40         50         60
[  372 ]    ____________******** i********s ********** *tt*svq**r a*tps*****>
  2.                                    30         40         50         60
[  243 ]    ______________________aqs*s*sip ********** *tt*svq**r a*tps*****>
                                                                          *p
                                                                           |
  3.              10         20         30         40         50         60 |
[  229 ]    ********** ********** i********s xrll*lvql* chhhlcarge sqh*icac*l>
                             *
                             |
```

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**FIGURE 10 (II)**

```
                        s|                                                    s
                        ||                                                    |
 5.           10        ||        30          40          50         60 |
[   74 ]    ******x*** ****smr**a q**s*-sipq tslig-pal- mppp*lckrr ep*lhlxlli>

                  190        200        210        220        230        240
                    *          *          *          *          *          *
MCG4        SRAPRPPASP GRPEQHTVIH MGNPEPLTHA PRKVYDTRDD DRTPGLHGDC DDDKYRRRPA
                                                     **
                                                      |
 1.           70         80         90        100 |  110        120
 [  372 ]    *q*****p** s********* **st*a*a** *******pgp *srhswetvm mtnt-aagl*>
 2.           70         80         90
[  243 ]    *q*****p** s********* **st*a*a** ***>
                                                      i
                                                      |
 3.         70         80         90        100 |  110        120
[  229 ]    gsp*sslpk* s*a-a*sht* gey*s*g*r- *kek*m*hg* ***a*i**** *******>
 4.          70         80         90        100        110        120
[   86 ]    __p*sslpk* s*a-a*sht* gey*s*g*rp kesi*h*gmm tgqqafm*** *********c>
                                                    h
```

**FIGURE 10 (III)**

```
                                                         |
  5.             70         80         90         100       110
[   74 ]     arl*allppq av*sstqsyt w*vlk*w-*t *qgk*m**** ***a*i**>
                                               g
                                               |
  6.                                           |100
[   38 ]     ____________________________*t *q*******>

                   250        260        270        280        290        300
                     *          *          *          *          *          *
MCG4         LGWLARLLRS RAGSRKRPLT LLQRAGLLLL LGLLGFLALL ALMSRLGRAA ADSDPNLDPL
  1.           130
[  372 ]     *****q**** ****>
  4.
[   86 ]     s*-**>

                   310
                     *
MCG4         MNPHIRVGPS
```

**FIGURE 10 (IV)**

Search Analysis for Sequence: MCG4

Search from 1 to 310

Date: September 22,1997

Matrix: pam250 matrix

Score Region from 1 to 310

Maximum possible score: 1598

Aligned sequences:

1. = EST AA074703 phase 1 translation
2. = EST AA134788 phase 3 translation
3. = EST AA134788 phase 2 translation
4. = EST AA074703 phase 3 translation
5. = EST AA074703 phase 2 translation
6. = EST AA134788 phase 1 translation

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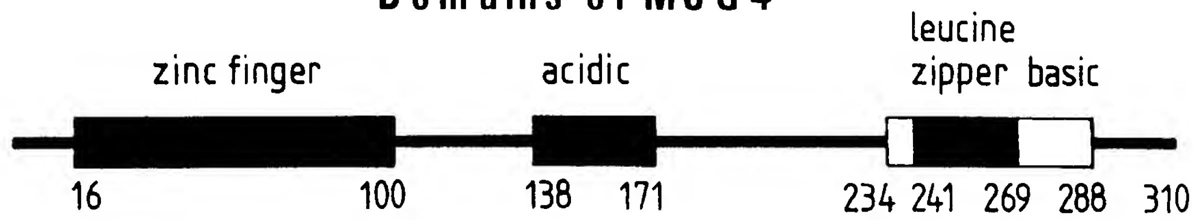
**FIGURE 11**

**Domains of MCG4**




zinc finger consensus: $CX_2HX_4CX_2CX_4HX_2CX_{17}CX_2CX_{18}HX_2CX_{18}CX_2C$

acidic domain consensus: 9/34 negatively charged amino acids, 0/34 positively charged

basic domain consensus: 13/55 positively charged amino acids, 0/55 negatively charged

leucine zipper domain consensus: $LX_6LX_6RX_6LX_6L$

alternate "novel" leucine zipper-like motif where leucine would not be aligned along the one surface of an alpha helix domain: (aa 261) $LX_6LXLX_6LXLX_6L$ (aa 286)

| | |
|---|---|
| FIG 12 (I) | FIG 12 (II) |
| FIG 12 (III) | FIG 12 (IV) |

FIG 12

FIG 12 (I)

Sequences producing High-scoring Segment Pairs:

| | |
|---|---|
| gnl\|PID\|e236178 | (Z70752 F25B3.3 [Caenorhabditis ele... |
| gi\|1293099 | (U53884) aimless RasGEF [Dictyosteli... |
| gi\|1655941 | (U67326) Ras-GRF2 [Mus musculus] |
| pir\|\|s30356 | CDC25 protein homolog - yeast (Cand... |
| sp\|P43069\|CC25_CANAL | CELL DIVISION CONTROL PROTEIN 25 |
| sp\|P28818\|GNRP_RAT | GUANINE NUCLEOTIDE RELEASING PROTEIN... |
| prf\|\|1814463A | guanine nucleotide-releasing factor ... |
| pir\|\|B46199 | nucleotide-exhange-factor homolog c... |
| gnl\|\|PID\|e238680 | (X97560) hypothetical protein L1309 ... |
| pir\|\|s22693 | CDC25 protein homolog - mouse/gi 50... |
| sp\|P14771\|SC25_ YEAST | SCD25 PROTEIN /gi 457494 (M26647) SD... |
| sp\|P26674\|STE6_SCHPO | STE6 PROTEIN /pir s28098 ste6 prote... |
| pir\|\|s28407 | CDC25 protein homolog - mouse |
| sp\|P27671\|GNRP_MOUSE | GUANINE NUCLEOTIDE RELEASING PROTEIN... |
| gi\|386047 | (s62035) Ras-specific guanine nucleo... |
| sp\|Q02342\|CC25_SACKL | CELL DIVISION CONTROL PROTEIN 25 /pi... |
| pir\|\|s14177 | SCD25 protein - yeast (Saccharomyces... |
| gi\|433720 | (L26584) CDC25 [Homo sapiens] |
| gnl\|PID\|e241744 | (Z68880) T14G10.2 [Caenorhabditis el... |
| gi\|3484 | (X03579) CDC25 protein (aa 1-1588) [... |

| High Score | smallest Sum Probability P(N) | N |
|---|---|---|
| 307 | 3.0e-124 | 8 |
| 202 | 7.8e-22 | 5 |
| 152 | 3.6e-16 | 4 |
| 150 | 2.2e-15 | 3 |
| 150 | 2.2e-15 | 3 |
| 166 | 2.6e-15 | 3 |
| 166 | 2.6e-15 | 3 |
| 167 | 1.1e-14 | 1 |
| 158 | 3.0e-14 | 3 |
| 167 | 3.7e-14 | 2 |
| 158 | 4.6e-14 | 3 |
| 160 | 5.2e-14 | 2 |
| 167 | 1.2e-13 | 3 |
| 167 | 1.2e-13 | 3 |
| 153 | 2.0e-13 | 2 |
| 142 | 4.5e-13 | 2 |
| 152 | 5.7e-13 | 3 |
| 153 | 6.0e-13 | 3 |

FIG 12 (II)

| | |
|---|---|
| sp\|P04821\|CC25_YEAST | CELL DIVISION CONTROL PROTEIN 25 /pi... |
| gi\|915328 | (U24070) Munc13-1 [Rattus norvegicus] |
| pir\|\|A46199 | nucleotide-exchange-factor homolog c... |
| pdb\|\|1PTR\| | Molecule: Protein Kinase C Delta Ty... |
| gi\|915330 | (U24071) Munc13-2 [Rattus norvegicus] |
| gi\|474982 | (D21239) 'C3G protein' [Homo sapiens... |
| gi\|1763306 | (U75361) Munc13-3 [Rattus norvegicus] |
| gi\|806957 | guanine-nucleotide exchange factor C... |
| sp\|Q03385\|GNDS_MOUSE | GUANINE NUCLEOTIDE DISSOCIATION STIM... |
| pir\|\|BVBYL1 | LTE1 protein - yeast (Saccharomyces... |
| gi\|452242 | (D21354) a putative guanine nucleoti... |
| sp\|P07866\|LTE1_YEAST | LOW TEMPERATURE ESSENTIAL PROTEIN /P... |
| gi\|509050 | (Z22521) protein kinase C delta [Hom... |
| gi\|520587 | (D10495) protein kinase C delta-type... |
| sp\|P05130\|KPC1_DROME | PROTEIN KINASE C, BRAIN ISOZYME (PKC... |
| pir\|\|S35704 | protein kinase C (EC 2.7.1.-) delta... |
| sp\|Q05655/KPCD_HUMAN | PROTEIN KINASE C, DELTA TYPE (NPKC-D... |
| pir\|\|S40279 | protein kinase C mu - human /pir A5... |
| sp\|P09215\|KPCD_RAT | PROTEIN KINASE C, DELTA TYPE (NPKC-D... |
| gi\|520878 | (Z34524) serine/threonine protein ki... |
| gi\|1519719 | (U68142) RalGDS-like [Homo sapiens] |

FIG 12 (III)

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| | | |
|---|---|---|
| 157 | 7.2e-13 | 1 |
| 136 | 3.4e-12 | 3 |
| 136 | 3.4e-12 | 3 |
| 151 | 5.5e-12 | 1 |
| 149 | 5.6e-12 | 1 |
| 136 | 1.5e-11 | 1 |
| 150 | 1.6e-11 | 2 |
| 131 | 3.3e-11 | 3 |
| 153 | 6.4e-11 | 2 |
| 128 | 7.8e-11 | 3 |
| 133 | 1.0e-10 | 2 |
| 139 | 1.9e-10 | 1 |
| 139 | 2.7e-10 | 1 |
| 139 | 2.7e-10 | 1 |
| 137 | 4.0e-10 | 1 |
| 137 | 4.6e-10 | 1 |
| 137 | 4.7e-10 | 1 |
| 137 | 4.7e-10 | 1 |
| 137 | 4.7e-10 | 1 |
| 137 | 4.9e-10 | 1 |
| 135 | 9.0e-10 | 1 |
| 133 | 1.8e-09 | 1 |
| 115 | 3.8e-09 | 3 |

FIG 12 IV

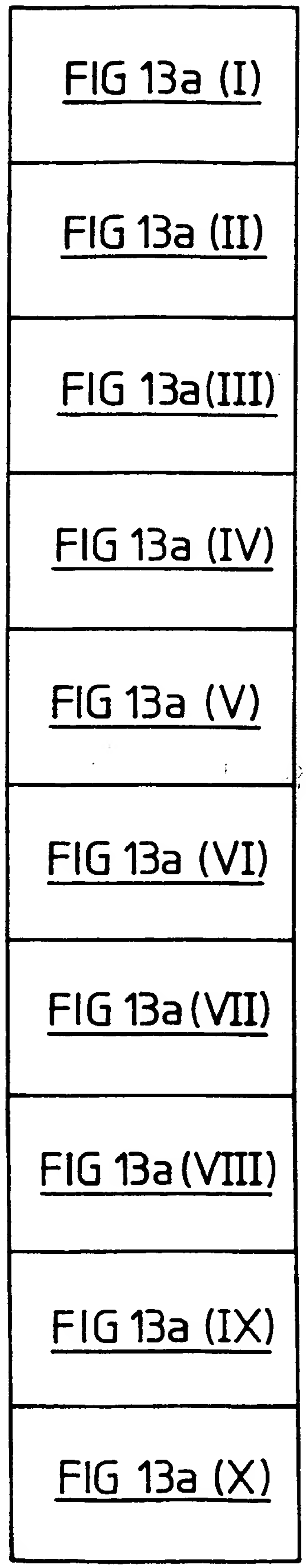
FIG 13a (I)
FIG 13a (II)
FIG 13a(III)
FIG 13a (IV)
FIG 13a (V)
FIG 13a (VI)
FIG 13a(VII)
FIG 13a(VIII)
FIG 13a (IX)
FIG 13a (X)

FIG 13a

**FIGURE 13(a) (I)**

```
CG ATT TCA TTC CTC GCT CCC CAC AGG TCC CTC TCC CCA AAA TAT   44
   Ile Ser Phe Leu Ala Pro His Arg Ser Leu Ser Pro Lys Tyr
    1               5                   10

TCC CAT CTT GTC CTA GCC CAT CCC CCA GAC TAT CTC AAG GAC CAG   89
Ser His Leu Val Leu Ala His Pro Pro Asp Tyr Leu Lys Asp Gln
 15                 20                  25

CTG TCC CCA CGC CCC CGA CCT CCA CTA GGC CTG TGC CAC CCG CTG  134
Leu Ser Pro Arg Pro Arg Pro Pro Leu Gly Leu Cys His Pro Leu
 30                 35                  40

CCT GCA GGA AGA CGC CCG GTC CCG GGC CGG GTT AGC CCC ATG GGA  179
Pro Ala Gly Arg Arg Pro Val Pro Gly Arg Val Ser Pro Met Gly
 45                 50                  55

ACG CAG CGC CTG TGT GGC CGC GGG ACT CAA GGC TGG CCT GGC TCA  224
Thr Gln Arg Leu Cys Gly Arg Gly Thr Gln Gly Trp Pro Gly Ser
 60                 65                  70

AGT GAA CAG CAC GTC CAG GAG GCG ACC TCG TCC GCG GGT TTG CAT  269
Ser Glu Gln His Val Gln Glu Ala Thr Ser Ser Ala Gly Leu His
 75                 80                  85
```

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**FIGURE 13(a) (II)**

```
TCT GGG GTG GAC GAG CTG GGG GTT CGG TCC GAG CCC GGT GGG AGG  314
Ser Gly Val Asp Glu Leu Gly Val Arg Ser Glu Pro Gly Gly Arg
 90                  95                 100

CTC CCG GAG CGC AGC CTG GGC CCA GCC CAC CCC GCG CCG GCG GCC  359
Leu Pro Glu Arg Ser Leu Gly Pro Ala His Pro Ala Pro Ala Ala
105                 110                 115

ATG GCA GGC ACC CTG GAC CTG GAC AAG GGC TGC ACG GTG GAG GAG  404
Met Ala Gly Thr Leu Asp Leu Asp Lys Gly Cys Thr Val Glu Glu
120                 125                 130

CTG CTC CGC GGG TGC ATC GAA GCC TTC GAT GAC TCC GGG AAG GTG  449
Leu Leu Arg Gly Cys Ile Glu Ala Phe Asp Asp Ser Gly Lys Val
135                 140                 145

CGG GAC CCG CAG CTG GTG CGC ATG TTC CTC ATG ATG CAC CCC TGG  494
Arg Asp Pro Gln Leu Val Arg Met Phe Leu Met Met His Pro Trp
150                 155                 160

TAC ATC CCC TCC TCT CAG CTG GCG GCC AAG CTG CTC CAC ATC TAC  539
Tyr Ile Pro Ser Ser Gln Leu Ala Ala Lys Leu Leu His Ile Tyr
165                 170                 175
```

**FIGURE 13(a) (III)**

```
CAA CAA TCC CGG AAG GAC AAC TCC AAT TCC CTG CAG GTG AAA ACG  584
Gln Gln Ser Arg Lys Asp Asn Ser Asn Ser Leu Gln Val Lys Thr
180                 185                 190

TGC CAC CTG GTC AGG TAC TGG ATC TCC GCC TTC CCA GCG GAG TTT  629
Cys His Leu Val Arg Tyr Trp Ile Ser Ala Phe Pro Ala Glu Phe
195                 200                 205

GAC TTG AAC CCG GAG TTG GCT GAG CAG ATC AAG GAG CTG AAG GCT  674
Asp Leu Asn Pro Glu Leu Ala Glu Gln Ile Lys Glu Leu Lys Ala
210                 215                 220

CTG CTA GAC CAA GAA GGG AAC CGA CGG CAC AGC AGC CTA ATC GAC  719
Leu Leu Asp Gln Glu Gly Asn Arg Arg His Ser Ser Leu Ile Asp
225                 230                 235

ATA GAC AGC GTC CCT ACC TAC AAG TGG AAG CGG CAG GTG ACT CAG  764
Ile Asp Ser Val Pro Thr Tyr Lys Trp Lys Arg Gln Val Thr Gln
240                 245                 250

CGG AAC CCT GTG GGA CAG AAA AAG CGC AAG ATG TCC CTG TTG TTT  809
Arg Asn Pro Val Gly Gln Lys Lys Arg Lys Met Ser Leu Leu Phe
255                 260                 265
```

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**FIGURE 13(a) (IV)**

```
GAC CAC CTG GAG CCC ATG GAG CTG GCG GAG CAT CTC ACC TAC TTG  854
Asp His Leu Glu Pro Met Glu Leu Ala Glu His Leu Thr Tyr Leu
270                 275                 280

GAG TAT CGC TCC TTC TGC AAG ATC CTG TTT CAG GAC TAT CAC AGT  899
Glu Tyr Arg Ser Phe Cys Lys Ile Leu Phe Gln Asp Tyr His Ser
285                 290                 295

TTC GTG ACT CAT GGC TGC ACT GTG GAC AAC CCC GTC CTG GAG CGG  944
Phe Val Thr His Gly Cys Thr Val Asp Asn Pro Val Leu Glu Arg
300                 305                 310

TTC ATC TCC CTC TTC AAC AGC GTC TCA CAG TGG GTG CAG CTC ATG  989
Phe Ile Ser Leu Phe Asn Ser Val Ser Gln Trp Val Gln Leu Met
315                 320                 325

ATC CTC AGC AAA CCC ACA GCC CCG CAG CGG GCC CTG GTC ATC ACA 1034
Ile Leu Ser Lys Pro Thr Ala Pro Gln Arg Ala Leu Val Ile Thr
330                 335                 340
```

**FIGURE 13(a) (V)**

```
CAC TTT GTC CAC GTG GCG GAG AAG CTG CTA CAG CTG CAG AAC TTC 1079
His Phe Val His Val Ala Glu Lys Leu Leu Gln Leu Gln Asn Phe
345                 350                 355

AAC ACG CTG ATG GCA GTG GTC GGG GGC CTG AGC CAC AGC TCC ATC 1124
Asn Thr Leu Met Ala Val Val Gly Gly Leu Ser His Ser Ser Ile
360                 365                 370

TCC CGC CTC AAG GAG ACC CAC AGC CAC GTT AGC CCT GAG ACC ATC 1169
Ser Arg Leu Lys Glu Thr His Ser His Val Ser Pro Glu Thr Ile
375                 380                 385

AAG CTC TGG GAG GGT CTC ACG GAA CTA GTG ACG GCG ACA GGC AAC 1214
Lys Leu Trp Glu Gly Leu Thr Glu Leu Val Thr Ala Thr Gly Asn
390                 395                 400

TAT GGC AAC TAC CGG CGT CGG CTG GCA GCC TGT GTG GGC TTC CGC 1259
Tyr Gly Asn Tyr Arg Arg Arg Leu Ala Ala Cys Val Gly Phe Arg
405                 410                 415

TTC CCG ATC CTG GGT GTG CAC CTC AAG GAC CTG GTG GCC CTG CAG 1304
Phe Pro Ile Leu Gly Val His Leu Lys Asp Leu Val Ala Leu Gln
420                 425                 430
```

**FIGURE 13(a) (VI)**

```
CTG GCA CTG CCT GAC TGG CTG GAC CCA GCC CGG ACC CGG CTC AAC 1349
Leu Ala Leu Pro Asp Trp Leu Asp Pro Ala Arg Thr Arg Leu Asn
435                 440                 445

GGG GCC AAG ATG AAG CAG CTC TTT AGC ATC CTG GAG GAG CTG GCC 1394
Gly Ala Lys Met Lys Gln Leu Phe Ser Ile Leu Glu Glu Leu Ala
450                 455                 460

ATG GTG ACC AGC CTG CGG CCA CCA GTA CAG GCC AAC CCC GAC CTG 1439
Met Val Thr Ser Leu Arg Pro Pro Val Gln Ala Asn Pro Asp Leu
465                 470                 475

CTG AGC CTG CTC ACG GTG TCT CTG GAT CAG TAT CAG ACG GAG GAT 1484
Leu Ser Leu Leu Thr Val Ser Leu Asp Gln Tyr Gln Thr Glu Asp
480                 485                 490

GAG CTG TAC CAG CTG TCC CTG CAG CGG GAG CCG CGC TCC AAG TCC 1529
Glu Leu Tyr Gln Leu Ser Leu Gln Arg Glu Pro Arg Ser Lys Ser
495                 500                 505
```

**FIGURE 13(a) (VII)**

```
TCG CCA ACC AGC CCC ACG AGT TGC ACC CCA CCA CCC CGG CCC CCG 1574
Ser Pro Thr Ser Pro Thr Ser Cys Thr Pro Pro Pro Arg Pro Pro
510                 515                 520

GTA CTG GAG GAG TGG ACC TCG GCT GCC AAA CCC AAG CTG GAT CAG 1619
Val Leu Glu Glu Trp Thr Ser Ala Ala Lys Pro Lys Leu Asp Gln
525                 530                 535

GCC CTC GTG GTG GAG CAC ATC GAG AAG ATG GTG GAG TCT GTG TTC 1664
Ala Leu Val Val Glu His Ile Glu Lys Met Val Glu Ser Val Phe
540                 545                 550

CGG AAC TTT GAC GTC GAT GGG GAT GGC CAC ATC TCA CAG GAA GAA 1709
Arg Asn Phe Asp Val Asp Gly Asp Gly His Ile Ser Gln Glu Glu
555                 560                 565

TTC CAG ATC ATC CGT GGG AAC TTC CCT TAC CTC AGC GCC TTT GGG 1754
Phe Gln Ile Ile Arg Gly Asn Phe Pro Tyr Leu Ser Ala Phe Gly
570                 575                 580

GAC CTC GAC CAG AAC CAG GAT GGC TGC ATC AGC AGG GAG GAG ATG 1799
Asp Leu Asp Gln Asn Gln Asp Gly Cys Ile Ser Arg Glu Glu Met
585                 590                 595
```

**FIGURE 13(a) (VIII)**

```
GTT TCC TAT TTC CTG CGC TCC AGC TCT GTG TTG GGG GGG CGC ATG 1844
Val Ser Tyr Phe Leu Arg Ser Ser Ser Val Leu Gly Gly Arg Met
600                 605                 610

GGC TTC GTA CAC AAC TTC CAG GAG AGC AAC TCC TTG CGC CCC GTC 1889
Gly Phe Val His Asn Phe Gln Glu Ser Asn Ser Leu Arg Pro Val
615                 620                 625

GCC TGC CGC CAC TGC AAA GCC CTG ATC CTG GGC ATC TAC AAG CAG 1934
Ala Cys Arg His Cys Lys Ala Leu Ile Leu Gly Ile Tyr Lys Gln
630                 635                 640

GGC CTC AAA TGC CGA GCC TGT GGA GTG AAC TGC CAC AAG CAG TGC 1979
Gly Leu Lys Cys Arg Ala Cys Gly Val Asn Cys His Lys Gln Cys
645                 650                 655

AAG GAT CGC CTG TCA GTT GAG TGT CGG CGC AGG GCC CAG AGT GTG 2024
Lys Asp Arg Leu Ser Val Glu Cys Arg Arg Arg Ala Gln Ser Val
660                 665                 670
```

**FIGURE 13(a) (IX)**

```
AGC CTG GAG GGG TCT GCA CCC TCA CCC TCA CCC ATG CAC AGC CAC 2069
Ser Leu Glu Gly Ser Ala Pro Ser Pro Ser Pro Met His Ser His
675                 680                 685

CAT CAC CGC GCC TTC AGC TTC TCT CTG CCC CGC CCT GGC AGG CGA 2114
His His Arg Ala Phe Ser Phe Ser Leu Pro Arg Pro Gly Arg Arg
690                 695                 700

GGC TCC AGG CCT CCA GAG ATC CGT GAG GAG GAG GTA CAG ACG GTG 2159
Gly Ser Arg Pro Pro Glu Ile Arg Glu Glu Glu Val Gln Thr Val
705                 710                 715

GAG GAT GGG GTG TTT GAC ATC CAC TTG TA ATAGATGCTG           2198
Glu Asp Gly Val Phe Asp Ile His Leu  *
720                 725

TGGTTGGATC AAGGACTCAT TCCTGCCTTG GAGAAAATAC TTCAACCAGA      2248

GCAGGGAGCC TGGGGGTGTC GGGGCAGGAG GCTGGGGATG GGGGTGGGAT      2293

ATGAGGGTGG CATGCAGCTG AGGGCAGGGC CAGGGCTGGT GTCCCTAAGG      2348
```

**FIGURE 13(a) (X)**

TTGTACAGAC TCTTGTGAAT ATTTGTATTT TCCAGATGGA ATAAAAAGGC 2398

CCGTGTAATT AACCTTC (A)n 2416

**FIGURE 13(b)**

```
CGATTTCATT CCTCGCTCCC CACAGGTCCC TCTCCCCAAA ATATTCCCAT      50
CTTGTCCTAG CCCATCCCCC AGACTATCTC AAGGACCAGC TGTCCCCACG     100
CCCCCGACCT CCACTAGGCC TGTGCCACCC GCTGCCTGCA GGAAGACGCC     150
CGGTCCCGGG CCGGGTTAG CCC CAT GGG AAC GGG GTT CGG TCC GAG   196
                   *  Pro His Gly Asn Gly Val Arg Ser Glu
                          1               5
CCC GGT GGG AGG CTC CCG GAG CGC AGC CTG GGC CCA GCC CAC    238
Pro Gly Gly Arg Leu Pro Glu Arg Ser Leu Gly Pro Ala His
 10                  15                  20
CCC GCG CCG GCG GCC ATG GCA GGC ACC CTG GAC CTG GAC AAG    280
Pro Ala Pro Ala Ala Met Ala Gly Thr Leu Asp Leu Asp Lys
     25                  30                  35
GGC TGC ACG GTG GAG GAG CT                                 300
Gly Cys Thr Val Glu Glu Leu
         40
```

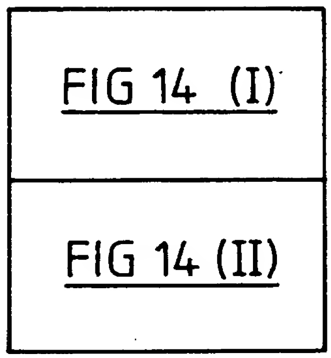


FIG 14

**FIGURE 14 (I)**

```
1     MAGTLDLDKGC...TVEELLRGCIEAF..DDSGKVRDPQLVRMFLMMHPW    45

1     MSSKVEEDQHQELLTEDQLVARCVECFDVDEEDEVEDIEFVDALFLSHQW    50

46    YIPSSQLAAKLLHIYQQSRKDNSNSLQVKTCHLVRYWISAFPAEFDLNPE    95

51    LSDSLSLITHFVNFYQETRNVEQRE...AVCRAVSFWIEKFPMHFDAQPQ    97

96    LAEQIKELKALLDQEGNRRHSSLIDIDSVPTYKWKRQVTQRNPVGQKK..   143

98    VCAQVVRLKTIAEDINENIRNGL.DVSALPSFAWLRAVSVRNPLAKQTIV   146

144   .........................RKMSLLFDHLEPMELAEHLTYLEYR   168

147   RVDFETLPTPGTPPPFPIASKKFSLTAFSLSFVQASPSDISTSLSHIDYR   196

169   SFCKILFQDYHSFVTHGCTVDNPVLERFISLFNSVSQWVQLMILSKPTAP   218

197   VLSTISITELKQYVKDGHLRSCPMLERSISVFNNLSNWVQCLILNKTTPK   246

219   QRALVITHFVHVAEKLLQLQNFNTLMAVVGGLSHSSISRLKETHSHVSPE   268

247   ERAEILVKFVHVAKHLRKINNFNTLMSVVGGITHSSVARLAKTYAVLSND   296

269   TIKLWEGLTELVTATGNYGNYRRRLAAC.VGFRFPILGVHLKDLVALQLA   317

297   IKKELTQLTNLLSAQHNFCEYRKALGACNKKFRIPIIGVHLKDLVAINCS   346
```

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**FIGURE 14 (II)**

```
318  LPDWLDPARTRLNGAKMKQLFSILEELAMVTSLRPPV.QANPDLLSLLTV  366
347  GANFEKT..KCISSDKLVKLSKLLSNFLVFNQKGHNLPEMNMDLINTLKV  394

367  SLDQYQTEDELYQLSLQREPRSKSSPTSPTSCTPPPRPPVLEEWTSAAKP  416
395  SLDIRYNDDDIYELSLRREPKTFMN.......FEPSRGLVFAEWASGVTV  437

417  KLDQALVVEHIEKMVESVFRNFDVDGDGHISQEEFQIIRGNFPYLSAFGD  466
438  APDNATVSKHISAMVDAVFKHYDHDRDGFISQEEFQLIAGNFPFIDAFVN  487

467  LDQNQDGCISREEMVSYFLRSS.SVLGGRMGFVHNFQESNSLRPVACRHC  515
488  IDVDMDGQISKDELKTYFMAANKNTKDLRRGFKHNFHETTFLTPTTCNHC  537

516  KALILGIYKQGLKCRACGVNCHKQCKDRLSVECRRRAQSVSLEGSAPSPS  565
538  NKLLWGILRQGFKCKDCGLAVHSCCKSNAVAECRRKSSSNLTRAAEWFAS  587

566  PMHSHHHRAFSFSLPRPGRRGSRPPEIREEEVQTVEDGVFDIHL  609
588  PRGSMRSRIINTC....NNSGSTPDEEIGLVSLACEEVFEDDDL  627
```

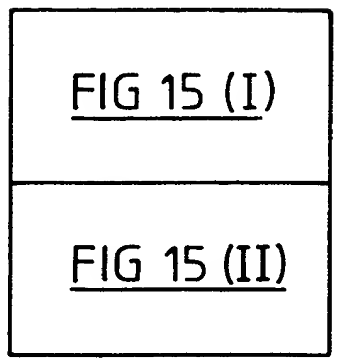



FIG 15

**FIGURE 15 (I)**

```
human CGATTTCATT CCTCGCTCCC CACAGGTCCC TCTCCCCAAA ATATTCCCAT CTTGTCCTAG  60
human CCCATCCCCC AGACTATCTC AAGGACCAGC TGTCCCCACG CCCCCGACCT CCACTAGGCC 120
human TGTGCCACCC GCTGCCTGCA GGAAGACGCC CGGTCCCGGG CCGGGTTAGC CCCATGGGAA 180
human CGCAGCGCCT GTGTGGCCGC GGGACTCAAG GCTGGCCTGG CTCAAGTGAA CAGCACGTCC 240
mouse                        ***tcag** ****ag**** t********* ***a*g***t>
human AGGAGGCGAC CTCGTCCGCG GGTTTGCATT CTGGGGTGGA CGAGCTGGGG GTTCGGTCCG 300
                                                      acagg
                                                        |
mouse g*****t**a **-*catt** ********** ***aa**aa* g**ct***** **a**aat**>
human AGCCCGGTGG GAGGCTCCCG GAGCGCAGCC TGGGCCCAGC CCACCCCGCG CCGGCGGCCA 360
mouse ***a*t**** *******tga ***t*t*a*t ****t*t*** ***-*tg**a *****a****>
human TGGCAGGCAC CCTGGACCTG GACAAGGGCT GCACGGTGGA GGAGCTGCTC CGCGGGTGCA 420
mouse ****ga**** t********* ********t* ****c***** ********** **t**c**t*>
human TCGAAGCCTT CGATGACTCC GGGAAGGTGC GGGACCCGCA GCTGGTGCGC ATGTTCCTCA 480
```

**FIGURE 15 (II)**

```
mouse **********  t********t  **a*******  *a**t**a**  ***a******  *****t****>
human TGATGCACCC  CTGGTACATC  CCCTCCTCTC  AGCTGGCGGC  CAAGCTGCTC  CACATCTACC 540
mouse **********  *********a  **t*******  *******tt*  g**a******  ***t****t*>
human AACAATCCCG  GAAGGACAAC  TCCAATTCCC  TGCAGGTGAA  AACGTGCCAC  CTGGTCAGGT 600
mouse *g********  **********  ********t*  *a***a****  ******t***  t*********>
human ACTGGATCTC  CGCCTTCCCA  GCGGAGTTTG  ACTTGAACCC  GGAGTTGGCT  GAGCAGATCA 660
mouse **********  a*********  **a*****c*  **********  a***c*****  **a*******>
human AGGAGCTGAA  GGCTCTGCTA  GACCAAGAAG  GGAACCGACG  GCACAGCAGC  CTAATCGACA 720
mouse **********  *******t**  **********  *******ca*  **********  **c*******>
human TAGACAGCGT                                                             730
mouse *c**g**t**
```

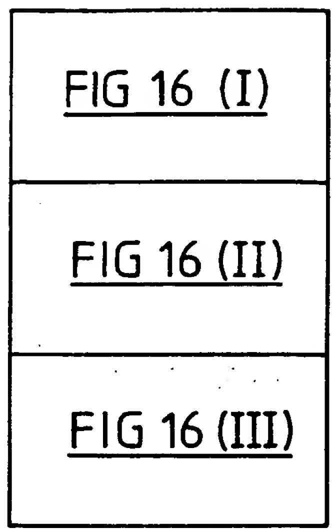



FIG 16

**FIGURE 16 (I)**

```
CACGCCTCGG AAGGGAGGTT TGGGGTCGGT GGTTTCACAG TGAGTGTGTC        50
TGAAGCCAAA TGGTCGGAAA CCGTTACCCG CTCTCCTAG GCC CGG CTA        98
                                         *  Ala Arg Leu
                                             1
GTG GGG ACC CCA ACC GCC TGC GGC TGC CCC TCC CAA GTT CCT      140
Val Gly Thr Pro Thr Ala Cys Gly Cys Pro Ser Gln Val Pro
     5                   10                  15

CCC TGT TGG CCA GGC ATC CAG GTC TCC AGT CTC CGA GCT GCG      182
Pro Cys Trp Pro Gly Ile Gln Val Ser Ser Leu Arg Ala Ala
         20                  25                  30

GAG AAC CCA CCG CCA CAT GCG GCT GCC CCT TTC CAT TCG ACC      224
Glu Asn Pro Pro Pro His Ala Ala Ala Pro Phe His Ser Thr
             35                  40                  45

CTG TGG GGA GCC AGG CTT CCG GGG CCC CGT TCC TCC TGT GTG      266
Leu Trp Gly Ala Arg Leu Pro Gly Pro Arg Ser Ser Cys Val
                 50                  55

AAC TGG GCC CCC CGC CCC CAT TCC CAG ACA TCA AGG CCG CGT      308
Asn Trp Ala Pro Arg Pro His Ser Gln Thr Ser Arg Pro Arg
 60                  65                  70
```

**FIGURE 16 (II)**

```
CTC CAG ATA GCC ACG ATT TCA TTC CTC GCT CCC CAC AGG TCC        350
Leu Gln Ile Ala Thr Ile Ser Phe Leu Ala Pro His Arg Ser
     75                  80                  85

CTC TCC CCA AAA TAT TCC CAT CTT GTC CTA GCC CAT CCC CCA        392
Leu Ser Pro Lys Tyr Ser His Leu Val Leu Ala His Pro Pro
         90                  95                  100

GAC TAT CTC AAG GAC CAG CTG TCC CCA CGC CCC CGA CCT CCA        434
Asp Tyr Leu Lys Asp Gln Leu Ser Pro Arg Pro Arg Pro Pro
            105                 110                 115

CTA GGC CTG TGC CAC CCG CTG CCT GCA GGA AGA CGC CCG GTC        476
Leu Gly Leu Cys His Pro Leu Pro Ala Gly Arg Arg Pro Val
                120                 125

CCG GGC CGG GTT AGC CCC ATG GGA ACG CAG CGC CTG TGT GGC        518
                 *  Pro His Gly Asn
Pro Gly Arg Val Ser Pro Met Gly Thr Gln Arg Leu Cys Gly
130                 135                 140
```

**FIGURE 16 (III)**

```
CGC GGG ACT CAA GGC TGG CCT GGC TCA AGT GAA CAG CAC GTC        560
Arg Gly Thr Gln Gly Trp Pro Gly Ser Ser Glu Gln His Val
    145                 150                 155

CAG GAG GCG ACC TCG TCC GCG GGT TTG CAT TCT GGG GTG GAC        602
Gln Glu Ala Thr Ser Ser Ala Gly Leu His Ser Gly Val Asp
        155                 160                 165

GAG CTG GGG GTT CGG TCC GAG CCC GGT GGG AGG CTC CCG GAG        644
Glu Leu Gly Val Arg Ser Glu Pro Gly Gly Arg Leu Pro Glu
            170                 175                 180

CGC AGC CTG GGC CCA GCC CAC CCC GCG CCG GCG GCC ATG GCA        686
Arg Ser Leu Gly Pro Ala His Pro Ala Pro Ala Ala Met Ala
                185                 190

GGC ACC CTG GAC CTG GAC AAG GGC TGC ACG GTG G                  720
Gly Thr Leu Asp Leu Asp Lys Gly Cys Thr Val
195                 200                 205
```

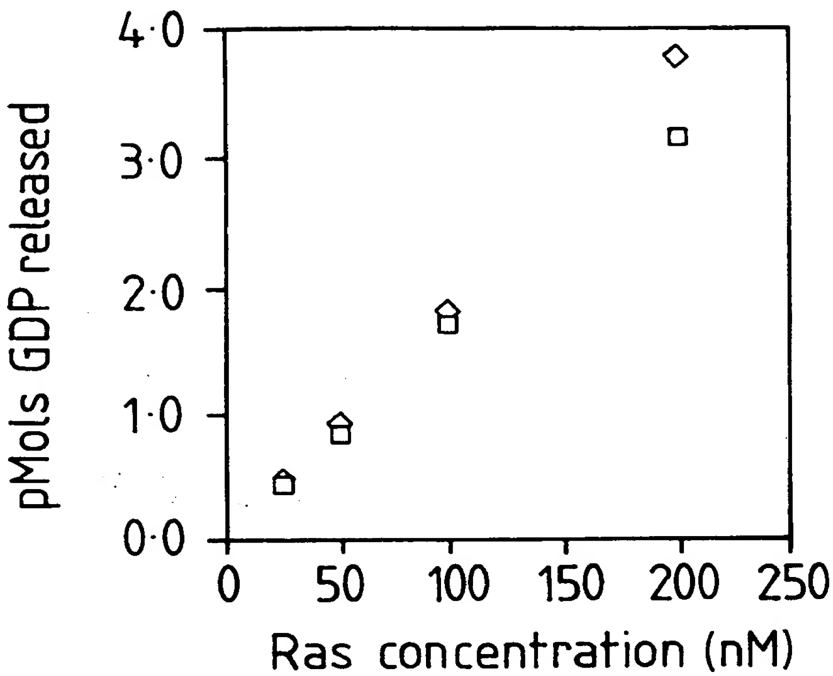
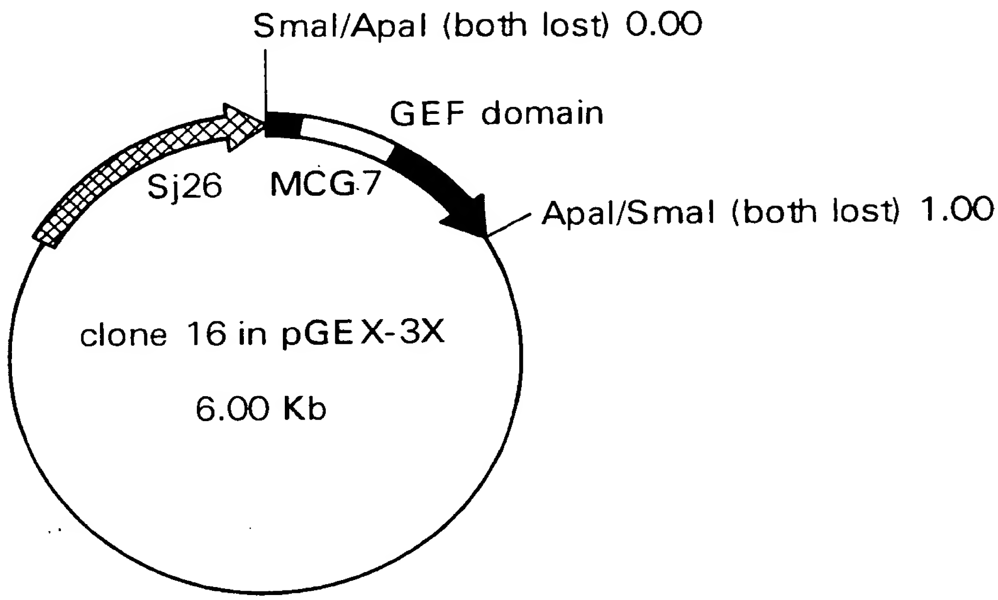

4·0
3·0
2·0
1·0
0·0
pMols GDP released
0
50
100
150
200
250
Ras concentration (nM)

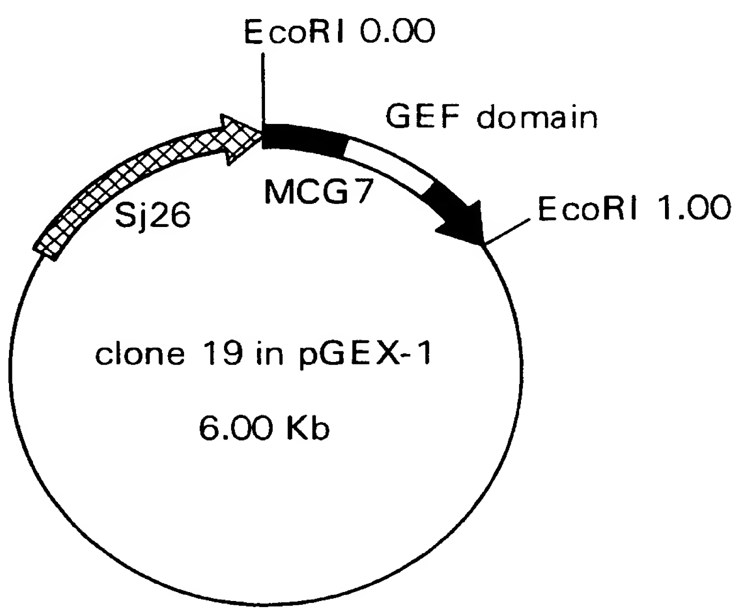
FIGURE 17

## FIGURE 18 (Cont. I)



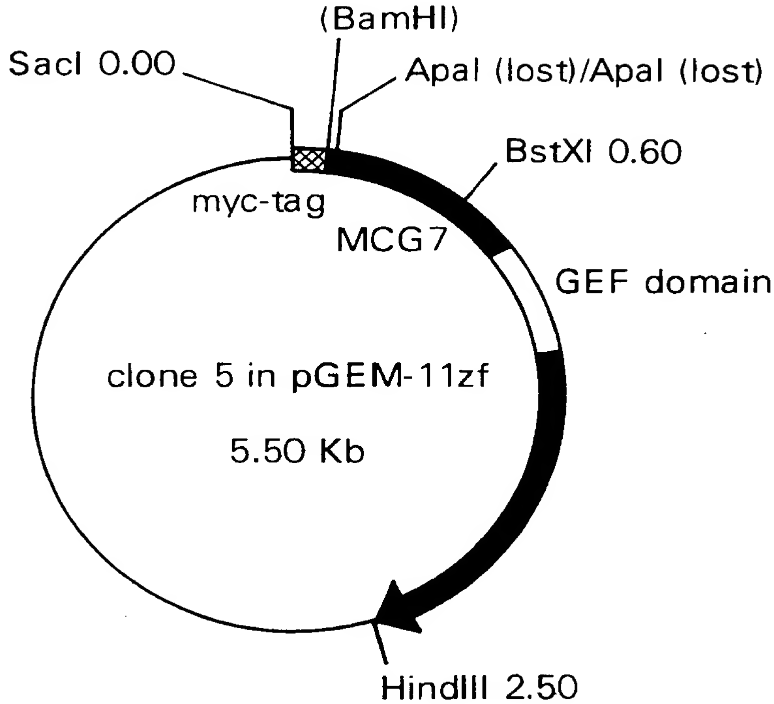

Plasmid name: clone 16 in pGEX-3X
Plasmid size:6.00 kb

## FIGURE 18 (Cont. II)

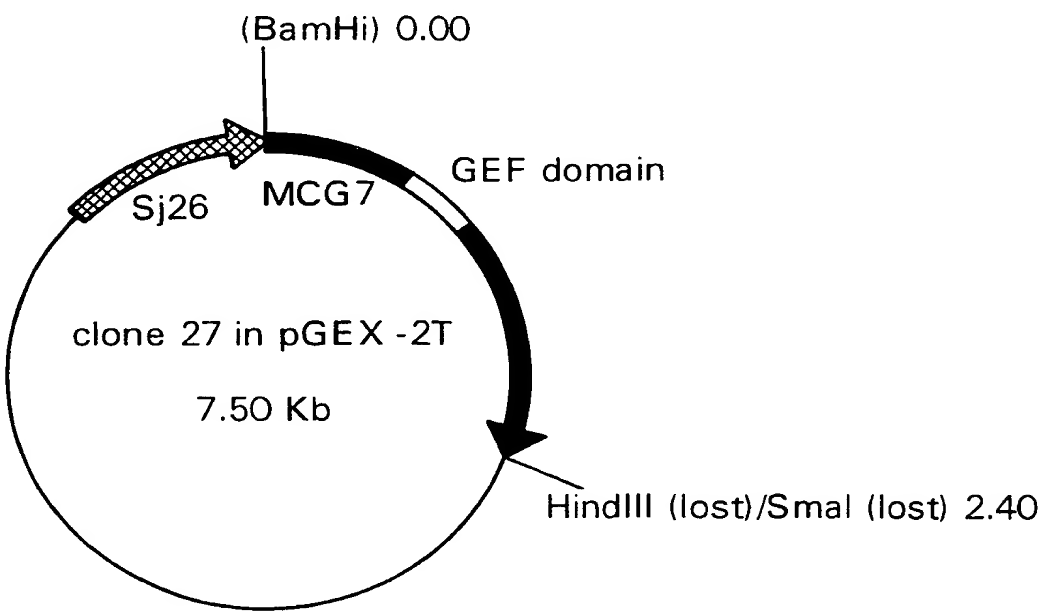



Plasmid name: clone 19 in pGEX-1
Plasmid size: 6.00 Kb

# FIGURE 18 (Cont. III)




Plasmid name: clone 5 in pGEM-11zf
Plasmid size: 5.50 kb




Plasmid name: clone 27 in pGEX-2T
Plasmid size: 7.50 kb

# FIGURE 18 (Cont. IV)

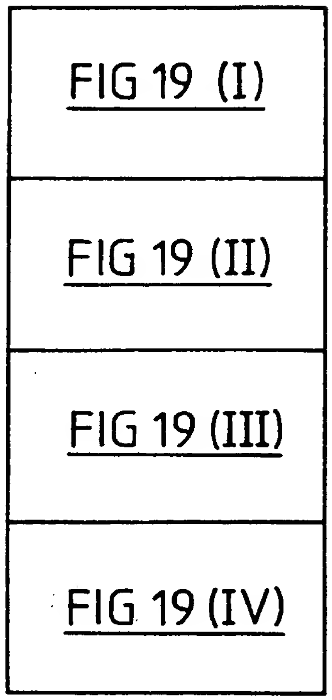



FIG 19

**FIGURE 19 (I)**

```
GCCCGCCGCC ATG CCG CCC TTA CTG CCC CTG CGC CTG TGC CGG       43
           Met Pro Pro Leu Leu Pro Leu Arg Leu Cys Arg
            1               5                   10

CTG TGG CCC CGC AAC CCT CCC TCC CGG CTC CTC GGA GCG GCC      85
Leu Trp Pro Arg Asn Pro Pro Ser Arg Leu Leu Gly Ala Ala
             15                  20                  25

GCC GGG CAG CGG TCC AGA CCC AGT ACT TAT TAT GAA CTG TTG     127
Ala Gly Gln Arg Ser Arg Pro Ser Thr Tyr Tyr Glu Leu Leu
                 30                  35

GGG GTG CAT CCT GGT GCC AGC ACT GAG GAA GTT AAA CGA GCT     169
Gly Val His Pro Gly Ala Ser Thr Glu Glu Val Lys Arg Ala
40                  45                  50

TTC TTC TCC AAG TCC AAA GAG CTG CAC CCA GAC CGG GAC CCT     211
Phe Phe Ser Lys Ser Lys Glu Leu His Pro Asp Arg Asp Pro
    55                  60                  65

GGG AAC CCA AGC CTG CAC AGC CGC TTT GTG GAG CTG AGC GAG     253
Gly Asn Pro Ser Leu His Ser Arg Phe Val Glu Leu Ser Glu
        70                  75                  80
```

**FIGURE 19 (II)**

```
GCA TAC CGT GTG CTC AGC CGT GAG CAG AGC CGC CGC AGC TAT      295
Ala Tyr Arg Val Leu Ser Arg Glu Gln Ser Arg Arg Ser Tyr
             85                  90                  95

GAT GAC CAG CTC CGC TCA GGT AGT CCC CCA AAG TCT CCA CGA      337
Asp Asp Gln Leu Arg Ser Gly Ser Pro Pro Lys Ser Pro Arg
                100                 105

ACC ACA GTC CAT GAC AAG TCT GCC CAC CAA ACA CAC AGC TCC      379
Thr Thr Val His Asp Lys Ser Ala His Gln Thr His Ser Ser
110                 115                 120

TGG ACA CCC CCC AAC GCA CAG TAC TGG TCC CAG TTT CAC AGC      421
Trp Thr Pro Pro Asn Ala Gln Tyr Trp Ser Gln Phe His Ser
    125                 130                 135

GTG AGG CCA CAG GGG CCC CAG TTG AGG CAG CAG CAA CAC AAA      463
Val Arg Pro Gln Gly Pro Gln Leu Arg Gln Gln Gln His Lys
        140                 145                 150

CAA AAC AAA CAA GTG CTG GGG TAC TGC CTC CTC CTC ATG CTG      505
Gln Asn Lys Gln Val Leu Gly Tyr Cys Leu Leu Leu Met Leu
            155                 160                 165
```

**FIGURE 19 (III)**

```
GCG GGC ATG GGC CTG CAC TAC ATT GCC TTC AGG AAG GTG AAG      547
Ala Gly Met Gly Leu His Tyr Ile Ala Phe Arg Lys Val Lys
                170                 175

CAG ATG CAC CTT AAC TTC ATG GAT GAA AAG GAT CGG ATC ATC      589
Gln Met His Leu Asn Phe Met Asp Glu Lys Asp Arg Ile Ile
180                 185                 190

ACA GCC TTC TAC AAC GAA GCC CGG GCA CGG GCC AGG GCC AAC      631
Thr Ala Phe Tyr Asn Glu Ala Arg Ala Arg Ala Arg Ala Asn
    195                 200                 205

AGA GGC ATC CTT CAG CAG GAG CGA CAA CGG CTA GGG CAG CGG      673
Arg Gly Ile Leu Gln Gln Glu Arg Gln Arg Leu Gly Gln Arg
        210                 215                 220

CAG CCG CCA CCA TCC GAG CCA ACC CAA GGC CCC GAG ATC GTG      715
Gln Pro Pro Pro Ser Glu Pro Thr Gln Gly Pro Glu Ile Val
            225                 230                 235

CCC CGG GGC GCC GGC CCC TGA GGGGCTC ACCTGGATGG GGCCTGCAGT    763
Pro Arg Gly Ala Gly Pro  *
                240
```

**FIGURE 19 (IV)**

GCGTTCCCGC TTTGCTTCCT TCCCTGGACG GCCCGCTCCC CGAAACGCGC 813

GCAATAAAGT GATTCGCAG 832

**FIGURE 20**

```
>sp|P08622|DNAJ_ECOLI DNAJ PROTEIN >pir ||HHECDJ heat shock protein
dnaJ -
     Escherichia coli >gi |145769 (M12565)-heat shock protein dnaJ
     [Escherichia coli] >gi |216441 (D10483) dnaJ protein
[Escherichia coli]
        Length = 376

    Score = 138 (63.7 bits), Expect = 1.2e-10, P = 1.2e-10
    Identities = 25/62 (40%), Positives = 39/62 (62%)

Query: 35 YYELLGVHPGASTEEVKRAFFSKSKELHPDRDPGNPSLHSRFVELSEAYRVLSREQSRRS 94
           YYE+LGV   A   E+++A+   + + HPDR+ G+     ++F E+ EAY VL+  Q R +
Sbjct:  6 YYEILGVSKTAEEREIRKAYKRLAMKYHPDRNQGDKEAEAKFKEIKEAYEVLTDSQKRAA 65
Query: 95 YD   96
           YD
Sbjct: 66 YD   67
```

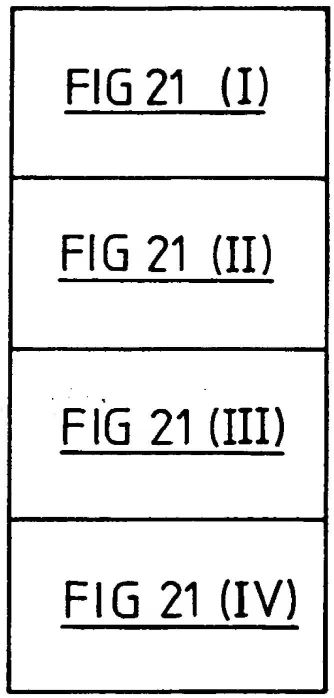



FIG 21

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**FIGURE 21 (I)**

```
>gi|1703590 (U80439) contains similarity to a DNAJ-like domain
(Caenorhabditis elegans]
        Length = 345

Score = 98 (45.2 bits), Expect = 5.2e-12, Sum P(3) = 5.2e-12
Identities = 17/37 (45%), Positives = 28/37 (75%)

Query: 28 QRSRPSTYYELLGVHPGASTEEVKRAFFSKSKELHPD 64
           ++ R  T+YE+LGV   A+   E+K AF+++SK++HPD
Sbjct: 22 KKIRQRTHYEVLGVESTATLSEIKSAFYAQSKKVHPD 58

Score = 74 (34.1 bits), Expect: = 5.2e-12, Sum P(3) = 5.2e-12
Identities = 17/32 (53%), Positives = 19/32 (59%)
```

**FIGURE 21 (II)**

```
Query: 71 SLHSRFVELSEAYRVLSREQSRRSYDDQLRSG 102
          S  + F+EL  AY VL R   RR YD QLR G
Sbjct: 64 SATASFLELKNAYDVLRRPADRRLYDYQLRGG 95

Score = 39 (18.0 bits), Expect = 5.2e-12, Sum P(3) = 5.2e-12
Identities = 10/42 (23%), Positives = 19/42 (45%)

Query: 162 LLMLAGMGLHYIAFRKVKQMHLNFMDEKDRIITAFYNEARAR 203
           L+++AG    Y+      Q L+  + ++D I   F  +   R
Sbjct: 158 LVLVAGYNGGYLYLLAYNQKQLDKLIDEDEIAKCFLRQKEFR 199
```

**FIGURE 21 (III)**

```
>gnl |PID |e281266 (Z81030) C01G10.12 [Caenorhabditis elegans]
        Length = 191
Score = 96 (44.3 bits), Expect = 1.8e-09, Sum P(3) = 1.8e-09
Identities = 17/41 (41%), Positives = 27/41 (65%)

Query: 35 YYELLGVHPGASTEEVKRAFFSKSKELHPDRDPGNPSLHSR 75
           YYE++GV   A+ +E++ AF  K+K+LHPD+   +    SR
Sbjct: 19 YYEIIGVSASATRQEIRDAFLKKTKQLHPDQSRKSSKSDSR 59

Score = 54 (24.9 bits), Expect = 1.8e-09, Sum P(3) = 1.8e-09
Identities = 10/22 (45%), Positives = 15/22 (68%)

Query: 75 RFVELSEAYRVLSREQSRRSYD 96
           +F+ + EAY VL  E+ R+ YD
```

**FIGURE 21 (IV)**

```
Sbjct: 71 QFMLVKEAYDVLRNEEKRKEYD 92

Score = 35 (16.1 bits), Expect = 1.8e-09, Sum P(3) = 1.8e-09
Identities = 9/44 (20%), Positives = 22/44 (50%)

Query: 141 QGPQLRQQQHKQNKQVLGYCLLLMLAGMGLHYIAFRKVKQMHLN 184
            + P+    + KQ  ++L      ++A +G + +  RK++   L+
Sbjct: 145 RNPEDEYLREKQKNRMLVVLAATVMALIGANIVYIRKLQADRLS 188
```

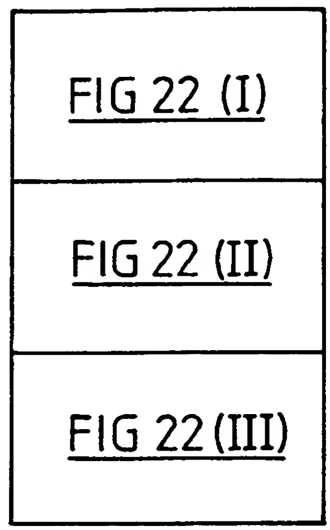


FIG 22

**FIGURE 22 (I)**

```
>sp|Q10209|YAY1_SCHPO HYPOTHETICAL 44.8 KD PROTEIN C4H3.01 IN
CHROMOSOME I
          >gi|1184014 (Z69380) unknown [Schizosaccharomyces pombe]
          Length = 392

Score = 84 (38.8 bits), Expect = 4.1e-08, Sum P(3) = 4.1e-08
Identities = 13/36 (36%), Positives = 25,36 (69%)

Query: 35 YYELLGVHPGASTEEVKRAFFSKSKELHPDRDPGNP 70
           YY+LLG+   A+  ++K+A+   + + HPD++P +P
Sbjct: 9  YYDLLGISTDATAVDIKKAYRKLAVKYHPDKNPDDP 44

Score = 64 (29.5 bits), Expect = 4.1e-08, Sum P(3) = 4.1e-08
Identities = 14/40 (35%), Positives = 23/40 (57%)
```

**FIGURE 22 (II)**

```
Query: 75 RFVELSEAYRVLSREQSRRSYDDQLRSGSPPKSPRTTVHD 114
          +F ++SEAY+VL  E+ R  YD   +  + P+   T  +D
Sbjct: 50 KFQKISEAYQVLGDEKLRSQYDQFGKEKAVPEQGFTDAYD 89

Score = 37 (17.1 bits), Expect = 4.1e-08, Sum P(3) = 4.1e-08
Identities = 9/29 (31%), Positives = 15/29 (51%)

Query: 190 DRIITAFYNEARARARANRGILQQERQRL 218
           DR   A   E  A A+  + +++  RQR+
Sbjct: 149 DRKKNAQIREREALAKREQEMIEDRRQRI 177

Score = 33 (15.2 bits), Expect = 0.00081, Sum P(3) = 0.00081

Identities = 8/19 (42%), Positives = 11/19 (57%)
```

**FIGURE 22 (III)**

```
Query: 140 PQGPQLRQQQHKQNKQVLG 158
           PQG   + Q+  +   QVLG
Sbjct: 44  PQGASEKFQKISEAYQVLG 62
```

# FIGURE 23

```
>gnl|PID|e253406 (X77635) tumorous imaginal discs [Drosophila virilis]
         >gnl|PID|e263866 (Y07700) Tid58 protein [Drosophila virilis]
         Length = 529

Score = 153 (70.6 bits), Expect = 9.7e-13, P = 9.7e-13
Identities = 27/71 (38%), Positives = 44/71 (61%)

Query: 26
        AGQRSRPSTYYELLGVHPGASTEEVKRAFFSKSKELHPDRDPGNPSLHSRFVELSEAYRV 85
        +  R +    YY  LGV   A+ +++K+A++  +K+ HPD +   +P     +F ++SEAY V
Sbjct: 72
        SSSRMQAKDYYATLGVAKNANAKDIKKAYYELAKKYHPDTNKDDPDASKKFQDVSEAYEV 131

Query: 86 LSREQSRRSYD 96
           LS +Q RR YD
Sbjct:132 LSDDQKRREYD 142
```

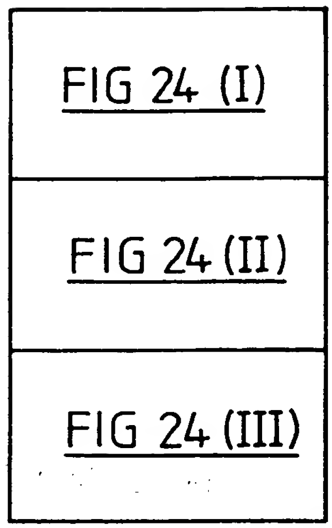


FIG 24

FIGURE 24(I)

```
MCG18   ---------------------MPPLLPLRLCRLWP-RN--PP-----------SRLLGAA
HDJ-2   MVKETTYYDVLGVKPNATQEELKKAYRKLALKYHPDKN--PN----EGEKFKQISQAYEV
HDJ-1   MGKD--YYQTLGLARGASDEEIKRAYRRQALRYHPDKNKEPG----AEEKFKEIAEAYDV
HSJ1    M-AS--YYEILDVPRSASADDIKKAYRRKALQWHPDKN--PDNKEFAEKKFKEVAEAYEV
                             .         .  * .*  *

MCG18   AGQRSRPSTY--YELLGVH-------------PGA------------ST-EEVKRAFFS-
HDJ-2   LSDAKKRELYDKGGEQAIK---------EGGAGGG----------FGSPMDIFDMFFGGG
HDJ-1   LSDPRKREIFDRYGEEGLKGSGP-----SGGSGGGANGTSFSYTFHGDPHAMFAEFFG--
HSJ1    LSDKHKREIYDRYGREGLTGTGTGPSRAEAGSGGP--G--FTFT-FRSPEEVFREFFG--
             .   .       .               *                 .   **

MCG18   KSKELHPDRDPGNP----SLHSRFVELSEAYRVLSREQSRRS--YDDQLRSGSPPKSPRT
HDJ-2   GRMQRERRGKNVVHQLSVTLEDLYNGATRKLALQKNVICDKCEGRGGKKGAVECCPNCRG
HDJ-1   GRNPFDTFFGQRNGEEGMDIDDPFSGFPMGMGGFTNVNFGRS--RSAQEPARKKQDPPVT
HSJ1    SGDPFAELFDDLGP--FSELQNRGSRHSGPFFTFSSSFPGHSDFSSSSFSFSPGAGAFRS
                           .                    ..
```

FIGURE 24 (II)

```
MCG18  TVHDKSAHQTHSSWTPPNAQY----WSQFHSVRPQ--------GP-----QLRQQQHKQN
HDJ-2  TGMQIRIHQIGPGMVQQIQSVCMECQGHGERISPK-DRCKSCNGRKIVREKKILEVHIDK
HDJ-1  HDLRVSLEEIYSGCTKKMK-----ISH-KRLNP---D------GKSIRNEDKILTIEVKK
HSJ-1  VSTSTTFVQGRRITTRRIME-----NGQ-ERVEVEED------GQ----LKSVTINGVPD
               .                  .   .           *

MCG18  KQVLGYCLLL-----MLAGMGLHYIAFRKVKQMHLNFMDE-KDRIITAFYNEARARARAN
HDJ-2  GMKDGQKITFHGEGDQEPGLEPGDIIIVLDQKDHAVFTRRGEDLFMCMDIQLVEALCGFQ
HDJ-1  GWKEGTKITFPKEGDQTSNNIPADIVFVLKDKPHNIFKRDGSDVIYPARISLREALCGCT
HSJ1   DLARGLELSR-RE--QQP-SVTSRSGGTQVQQTPASCPLD-SDLSEDEDLQLAMAYSLSE
           *  .                       .          *           * .

MCG18  RGILQQERQRLGQRQPP-PSEPTQGPEIVPRGAGP-------------------------
HDJ-2  KPISTLDNRTIVITSHPGQIVKHGDIKCVLNEGMPIYRRPYEKGRLIIEFKVNFPENGFL
HDJ-1  VNVPTLDGRTIPVVFK--DVIRPGMRRKVPGEGLPLPKTPEKRGDLIIEFEVIFPER--I
HSJ1   MEAAGKKPAGGREAQHR-RQGRPRPSTKIQAWGGP--RR--VRG--VKQPNAVHPQR-RR
                                   .     *
```

FIGURE 24 (III)

```
MCG18     ------------------------------------------------------------
HDJ-2     SPDKLSLLEKLLPERKEVEETDEMDQVELVDFDPNQERRRHYNGEAYEDDEHHPRGGVQC
HDJ-1     PQTSRTVLEQVLPI
HSJ1      PLAASSSEHRAQPD---------LIQILTGGSDSLWEEKRGVS-----------------

MCG18     ---
HDJ-2     QTS
HDJ-1     ---
HSJ1      ---
```

* = amino acid identity in all 4 proteins

\- = conservative substitution

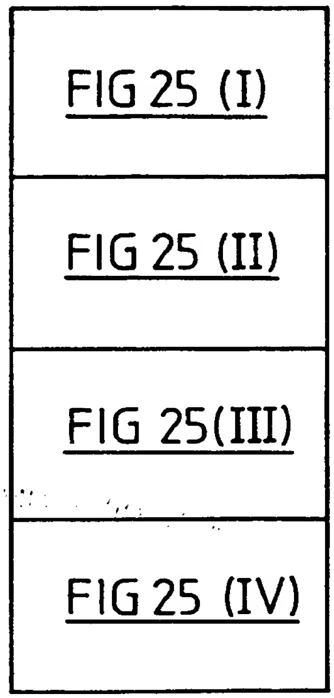


FIG 25

**FIGURE 25 (I)**

```
CAAGGAGCCT CTGCCTGCCC GTCGTCGTC ATG CCG TCC CTG TTG CTC         47
                                Met Pro Ser Leu Leu Leu
                                  1               5

CAG CTG CCC CTG CGC CTA TGC CGG CTG TGG CCG CAT AGC CTT         89
Gln Leu Pro Leu Arg Leu Cys Arg Leu Trp Pro His Ser Leu
             10                  15                  20

TCC ATC CGA CTT CTC ACA GCC GCC ACA GGG CAG CGG TCT GTC        131
Ser Ile Arg Leu Leu Thr Ala Ala Thr Gly Gln Arg Ser Val
                 25                  30

CCT ACT AAT TAC TAT GAA TTG TTG GGC GTG CAT CCG GGT GCC        173
Pro Thr Asn Tyr Tyr Glu Leu Leu Gly Val His Pro Gly Ala
 35                  40                  45

AGC GCT GAA GAG ATT AAA CGT GCT TTT TTC ACC AAG TCA AAA        215
Ser Ala Glu Glu Ile Lys Arg Ala Phe Phe Thr Lys Ser Lys
     50                  55                  60

GAG CTA CAC CCT GAT CGA GAC CCT GGG AAC CCA GCC CTG CAT        257
Glu Leu His Pro Asp Arg Asp Pro Gly Asn Pro Ala Leu His
         65                  70                  75
```

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**FIGURE 25 (II)**

```
AGC CGC TTT GTG GAG CTG AAT GAG GCA TAT CGA GTG CTC AGT      299
Ser Arg Phe Val Glu Leu Asn Glu Ala Tyr Arg Val Leu Ser
             80                  85                  90

CGT GAG GAA AGT CGT CGT AAC TAT GAC CAC CAG CTG CAT TCA      341
Arg Glu Glu Ser Arg Arg Asn Tyr Asp His Gln Leu His Ser
                 95                  100

GCC AGT CCT CCA AAG TCT TCA GGG AGC ACA GCC GAG CCT AAG      383
Ala Ser Pro Pro Lys Ser Ser Gly Ser Thr Ala Glu Pro Lys
105                 110                 115

TAT ACG CAA CAG ACA CAC AGC AGC TCC TGG GAA CCC CCC AAC      425
Tyr Thr Gln Gln Thr His Ser Ser Ser Trp Glu Pro Pro Asn
    120                 125                 130

GCT CAA TAC TGG GCC CAG TTC CAC AGT GTG AGG CCG CAG GGG      467
Ala Gln Tyr Trp Ala Gln Phe His Ser Val Arg Pro Gln Gly
        135                 140                 145
CCG GAG TCA AGG AAG CAG CAG CGT AAA CAC AAC CAG CGG GTC      509
Pro Glu Ser Arg Lys Gln Gln Arg Lys His Asn Gln Arg Val
            150                 155                 160
```

**FIGURE 25 (III)**

```
CTG GGG TAC TGC CTC CTG CTC ATG GTG GCA GGC ATG GGC CTG      551
Leu Gly Tyr Cys Leu Leu Leu Met Val Ala Gly Met Gly Leu
                165                     170

CAC TAT GTT GCC TTC AGG AAG CTG GAG CAG GTG CAT CGC AGC      593
His Tyr Val Ala Phe Arg Lys Leu Glu Gln Val His Arg Ser
175                 180                     185

TTC ATG GAT GAA AAG GAC CGG ATC ATT ACA GCC ATC TAC AAT      635
Phe Met Asp Glu Lys Asp Arg Ile Ile Thr Ala Ile Tyr Asn
    190                 195                     200

GAC ACT CGG GCC AGG GCC AGG GCC AAC AGA GCC AGG ATT CAG      677
Asp Thr Arg Ala Arg Ala Arg Ala Asn Arg Ala Arg Ile Gln
        205                     210                     215

CAG GAG CGC CAC GAG AGG CAG CAG CCT CGG GCA GAA CCC TCC      719
Gln Glu Arg His Glu Arg Gln Gln Pro Arg Ala Glu Pro Ser
            220                     225                     230
```

**FIGURE 25 (IV)**

```
CTG CCT CCA GAA AGC TCC AGG ATC ATG CCC CAG GAC ACA AGC      761
Leu Pro Pro Glu Ser Ser Arg Ile Met Pro Gln Asp Thr Ser
                235                 240

CCC TGAGAGGCTT AACTAAATGG GACCTTCATT GGTCCTCTCC CTGCTGCCTG   814
Pro  *
245

TCCAGAACTA CACGTGCAAT AAACTCATTT TCAG (A)n                    849
```

**FIGURE 26**

```
human MCG18 MPPLL---PLRLCRLWPRNPPSRLLGAAAGQRSRPSTYYELLGVHPGASTEEVKRAFFSK
mouse MCG18 MPSLLLQLPLRLCRLWPHSLSIRLLTAATGQRSVPTNYYELLGVHPGASAEEIKRAFFTK
            **      *********.    *** **.**** *..************.**.*****.*

human MCG18 SKELHPDRDPGNPSLHSRFVELSEAYRVLSREQSRRSYDDQLRSGSPPKSPRTTVHDKSA
mouse MCG18 SKELHPDRDPGNPALHSRFVELNEAYRVLSREESRRNYDHQLHSASPPKSSGSTAEPKYT
            *************.******** *********.*** ** **.* *****  .*   * .

human MCG18 HQTHSS-WTPPNAQYWSQFHSVRPQGPQLRQQQHKQNKQVLGYCLLLMLAGMGLHYIAFR
mouse MCG18 QQTHSSSWEPPNAQYWAQFHSVRPQGPESRKQQRKHNQRVLGYCLLLMVAGMGLHYVAFR
            .***** * *******.**********. *.**.*.*..*********.*******.***

human MCG18 KVKQMHLNFMDEKDRIITAFYNEARARARANRGILQQERQRLGQRQPPPSEPTQGPE---
mouse MCG18 KLEQVHRSFMDEKDRIITAIYNDTRARARANRARIQQER---HERQQPRAEPSLPPESSR
            *. *.*  *********** **..********  .****    .** * .**.  **

human MCG18 IVPRGAGP
mouse MCG18 IMPQDTSP
            *.*. . *
```

**FIGURE 27**

```
TTGA AGT CTA GCC CCA TCC TGG TCC AAT GCG CTC TTG GTA        40
  *  Ser Leu Ala Pro Ser Trp Ser Asn Ala Leu Leu Val
      1               5                   10
GCC TCC TTT CCC AGC TGC CCG CCC GCC GCC ATG CCG CCC TTA     82
Ala Ser Phe Pro Ser Cys Pro Pro Ala Ala Met Pro Pro Leu
        15                  20                  25

CTG CCC CTG CGC CTG TGC CGG CTG TGG CCC CGC AAC CC         120
Leu Pro Leu Arg Leu Cys Arg Leu Trp Pro Arg Asn Pro
            30                  35
```

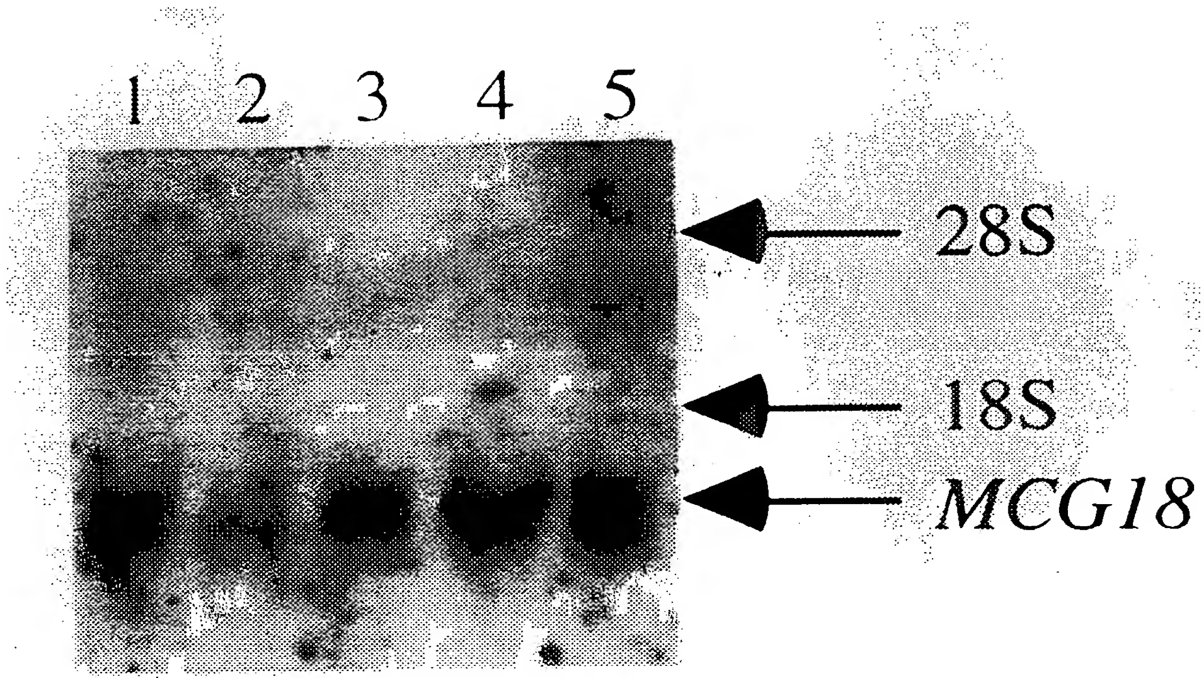


FIG 28

# INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00380

| A. | CLASSIFICATION OF SUBJECT MATTER |
|---|---|
| Int Cl[6]: | C12N 15/12; C07K 14/47; C07K 16/18; G01N 33/53 |

According to International Patent Classification (IPC) or to both national classification and IPC

| B. | FIELDS SEARCHED |
|---|---|

Minimum documentation searched (classification system followed by classification symbols)
I/C: WPAT (D gene) Sequences provided by Applicant

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
:EMBL, Genebank, Swiss Prot and PIR: Sequences provided by applicant
:

| C. | DOCUMENTS CONSIDERED TO BE RELEVANT | |
|---|---|---|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| P,X | Kedra D, Seroussi E, Fransson I, Trifunovic J, Clark M, Lagercranz J, Blennow E, Mehlin H, Dumanski J, Human Genetics, October 1997 100(5-6) 611-619 The germinal centre kinase gene and a novel CDC25-like gene are located in the vicinity of the PYGM gene on 11q13<br>EMBL AC Y12339 | 1-3,8-10,15-18 |
| P,X | Guru S C, Agarwal S K, Manickain P, Olufemi S E, et al Genome Research, July 1997 7(7) 725-735. A transcript map for the 2.8-Mb region containing the multiple endocrine neoplasia type I locus<br>TREMBL AC 014616 | 1. 4-5, 8, 11-12, 15, 19-21 |

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 16 July 1998 | 20 JUL 1998 |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200<br>WODEN ACT 2606<br>AUSTRALIA<br>Facsimile No.: (02) 6285 3929 | Authorized officer<br><br>GILLIAN ALLEN<br><br>Telephone No.: (02) 6283 2266 |

# INTERNATIONAL SEARCH REPORT

International Application No.
**PCT/AU 98/00380**

**Box 1** **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1, 2, 4, 6
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

They are to known groups of proteins and lack distinguishing features which would enable a meaningful search.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box II** **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Invention 1, defined by claims 2, 3, 9, 10, 16-18, is to nucleotide sequences, amino acid sequences and proteins with a zinc finger domain.
Invention 2, defined by claims 4, 5, 11, 12, 19-21, is to nucleotide sequences and amino acid sequences and proteins which are guanine exchange factors.
Invention 3, defined by claims 6, 7, 13, 14, 22-24, is to nucleotide sequences and amino acid sequences and proteins which are heat shock proteins or heat shock binding proteins.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest** ☐ The additional search fees were accompanied by the applicant's protest.
☒ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00380

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | EMBL AC AF012106<br>DT 6 November 1997<br>Lloyd S E and Thakker R V DE<br>Homo Sapiens DnaJ protein ($HSPF_2$)mRNA, complete cds | 1,6-8,13-15,22-24 |
| P,X | EMBL AC AF 036875<br>DT 20 May 1998<br>Silins G, Grimmond S, Hayward N DE<br>Mus musculus multiple endocrine neoplasia type I candidate protein number 18 mRNA, complete cds | 1,6-8,13-15,22-24 |

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

RECEIVED 13 JUL 1999 WIPO PCT

| Applicant's or agent's file reference 2049081/EJH | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416). |
|---|---|---|
| International application No. **PCT/AU** 98/00380 | International filing date *(day/month/year)* 22 May 1998 | Priority Date *(day/month/year)* 23 May 1997 |
| International Patent Classification (IPC) or national classification and IPC **Int. Cl.$^6$** C12N 15/12; CO7K 14/47 | | |
| Applicant AMRAD Operations Pty Ltd | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

| | | |
|---|---|---|
| I | ☒ | Basis of the report |
| II | ☐ | Priority |
| III | ☐ | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| IV | ☒ | Lack of unity of invention |
| V | ☒ | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| VI | ☐ | Certain documents cited |
| VII | ☐ | Certain defects in the international application |
| VIII | ☒ | Certain observations on the international application |

| Date of submission of the demand<br>17 December 1998 | Date of completion of the report<br>6 July 1998 |
|---|---|
| Name and mailing address of the IPEA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200<br>WODEN ACT 2606<br>AUSTRALIA<br>Facsimile No. (02) 6285 3929 | Authorized Officer<br><br>**GILLIAN ALLEN**<br>Telephone No. (02) 6283 2266 |

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INTERNATIONAL PREL ARY EXAMINATION REPORT

International application No.
**PCT/AU 98/00380**

**I. Basis of the report**

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/fig

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*
** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

The Application is to three separate proteins or groups of proteins.

Claims 2, 3, 9, 10, 16-18 are to zinc finger proteins or their encoding nucleic acid sequences.

Claims 4, 5, 11, 13,19-21 are to guanine exchange factor proteins or their encoding nucleic acid sequences.

Claims 6, 7, 13, 14, 22-24 are to heat shock or heat shock binding proteins.

There is no sequence homology between the three protein types

The only unifying feature is their function as gene regulatory proteins. However, gene regulatory proteins of all three types are known. It is also known that gene regulatory proteins can exert their effects via a variety of mechanisms. Therefore, this feature does not provide unity according to Rule 13.2 of the PCT

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Claims **3, 5, 7, 10, 12. 14-24** | **YES** |
| | Claims **1, 2, 4, 6, 8, 9, 11, 13** | **NO** |
| Inventive step (IS) | Claims **3, 5, 7, 10, 12. 14-24** | **YES** |
| | Claims **1, 2, 4, 6, 8, 9, 11, 13** | **NO** |
| Industrial applicability (IA) | Claims **1-24** | **YES** |
| | Claims | **NO** |

2. Citations and explanations (Rule 70.7)

Citations

There was no close prior art found in the International Search to the claims searched. However, claims 1, 2, 4, and 6 were not searched

Novelty and Inventive Step

Claims1, 2, 4, 6, 8, 9, 11, 13 lack novelty and inventive step over the common general knowledge of the art and the disclosures of the description.

Claims 1 and 8 are to any protein regulator of gene expression. It is well known to anyone skilled in the art that gene expression can be regulated by proteins, and very many such proteins are known and have been sequenced. Therefore these claims lack novelty over the common general knowledge of the art.

Claims 2 and 9 are to $(HC_3)_2$ type zinc finger proteins. Zinc fingers are well known structural domains or motifs which bind to DNA. Figs 4-6 of the description disclose the structure of the zinc finger motif and zinc finger proteins, homolgous to MCG4, from *C. elegans* and *Saccharomyces pombe*. Therefor claims 1, 2, 8 and 9 lack novelty over these disclosures

Claims 4 and 11 are to guanine exchange factors (GEFs). This is a well known group of proteins which include the Ras oncogene.
Fig 12 discloses a number of known GEFs. Thus clains 1, 4, 8 and 11 lack novelty over these disclosures.

Claims 6 and 13 are to heat shock or heatshock binding proteins. Once again these are an extremely well known group of proteins. Figures 20 and 24 disclose DnaJ proteins, a type of heat shock protein. Thus claims 1, 6, 8 and 13 are not novel over these disclosures.

There is no close prior art which discloses the nucleic or amino acid sequences of the proteins designated MCG4, MCG7 or MCG18. Therefore claims 3, 5, 7, 10, 12 and 14-24 are considered novel and inventive.

## VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 3, 5, 7, 10, 12 and 14, parts (iii) and (iv) of each, are to nucleic acid sequences with at least 40% homology to, or which hybridise at low stringency with the nucleic acid sequences specified in the claims. It is considered that the description does not fully support claims to sequences having such low homology to those disclosed in the description. Nor does it support nucleic acid sequences which encode proteins of different functions to MCG4, MCG7 or MCG18, or which do not encode any polypeptide.

Claims 3, 5, 7, 10, 12 and 14, part (iv) are not clear. They place no limit on the length of the hybridising sequences, so the scope of the claims is indeterminate.